CYTOGENETIC STUDIES ON PENTACHLOROPHENOL (PCP) AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) STRESSED MICE (*MUS MUSCULUS*)

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

Master of Philosophy

IN

Zoology

RUBEENA SABIR

SECTION OF GENETICS
DEPARTMENT OF ZOOLOGY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2003

DEDICATED
TO MY FAMILY

Aligarh Muslim University, Aligarh 202 002 (U.P.)-India
Department of Zoology

GENETOX

Dr Waseem Ahmad (Faridi)
Incharge, Genetics Division
Gene-Tox Lab

Certificate

I certify that the work entitled " **Cytogenetic studies on Pentachlorophenol (PCP) and 2,4-Dichlorophenoxyacetic Acid (2,4-D) stressed mice (*Mus musculus*)"** is completed under my supervision by Miss Rubeena Sabir. The work is original and independently carried by the candidate herself. It embodies some of the interesting findings and add to the existing knowledge on the present topic.

I allow her to submit the dissertation for the degree of Master of Philosophy.

..... **(Dr.Waseem Ahmad Faridi)**
Supervisor

ACKNOWLEDGEMENTS

I would like to pay my gratitude to Professor Durdana Jairajpuri, chairperson, Department of Zoology for the Lab facility and academic support.

I am deeply and sincerely grateful to my Supervisor, Dr. Waseem Ahmad (Faridi) who has been guiding me throughout my research work. Without his able guidance, this work could not have been completed.

I thank my lab colleagues, Dr. Niamat Ali, Dr. Abul Farah, Dr. Bushra Ateeq and Mr. Faisal Siddiqui for their active co-operation.

I express my sincere gratitude to senior faculty members of the Department Prof. Jamil A. Khan, Prof. A. K. Jafri, Prof. Shamim Jairajpuri, Prof. Irfan Ahmad and Dr. Shahid Siddiqui.

My sincere gratitude to Faculty of Genetics Section Dr. Mohd. Afzal, Dr. Absarul Hasnain, Dr. Anjum Ara and Dr. Asim Azfer.

I owe my sincere thanks to my friends, Mr. Abbas Haider, Mr. Kashif Bieg, Mr. Navneet Sameul and Ms. Ayesha for their help and moral support.

I also thank Mr. Ashfaq Ahmad for computer analysis of my statistical calculations.

I would like to pay my sincere gratitude to Global Service, Rafeeq complex for all their typing, printing facilities, and their stoic demeanour.

I express my sincere gratitude to my family member's for their all support, and love.

In last but not least I thank Almighty "ALLAH" for His shower of kind benediction.

CONTENTS

LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
2,4 DB	2,4 Dichlorophenoxy butyric acid
2,4 DEP	2,4 Dichlorophenoxy ethyl phosphate
2,4D	2,4 Dichlorophenoxy acetic acid
C	Centigrade
CA	Chromosomal Aberration
CCT	Canadian center of toxicology
CHO	Chinese Hamster Ovary
DMSO	Dimethyl Sulpho Oxide
DNA	Deoxyribo Nucleic Acid
DPX	Distyrene Plsticizer Xylene
DW	Distilled Water
EEC	European Economic Community
EPA	Environmental Protection Agency
EPAFI	Environmental Protection Agency Federal Insecticide
EPAPAG	Environmental Protection Agency Pesticide Assessment Guideline.
gm	gram
H_2O	Water
hr	hour
IARC	International Agricultural Research Council
IARCGAP	International Agency for Research on Cancer
KCl	Potassium Chloride

KH_2PO_4	Potassium Phosphate Monobasic
LD	Lethal Dose
M	Molar
MAFF	Ministry of Agriculture, Forestry and Fisheries
MCPB	4 (4-chloro-o-tolyloxy) butric acid
MNCEs	Micronucleated Normochromatic Erythocytes
MPCEs	Micronucleated Polychromatic Erylonocytic
NTP	National Toxicological Programme
OECD	Organisation for Economic Corporation and Development
ppm	parts per million
SCE	Sister Chromatid Exchange
TEPA	Tris-azaridinyl-phosphine-oxide
TMP	Trimethylphospate

LIST OF TABLES

LIST OF FIGURES

time intervals in *Mus musculus*.

Figure 8: Multiple concentrations and durations dependent profiles of mitotic index (MI) by 2,4 Dichlorophenoxy acetic Acid (2,4-D) at different time intervals in *Mus musculus*.

Figure 9: Representative Photomicrograph of normal karyotype (**a**) and chromosomal aberrations (**b**, **c** and **d**) observed in bone marrow cells of *Mus musculus* by PCP and 2,4-D.

Figure 10: Representative Photomicrographs of micronucleus formation in polychromatic erythrocytes in bone marrow cells of *Mus musculus* by PCP and 2,4-D.

CHAPTER

Introduction and Review of Literature
Review of literature on 2,4-D
Review of litrature on PCP

INTRODUCTION AND REVIEW OF LITERATURE

Genetic toxicology has been greatly benefitted by various cytogenetic techniques that mainly deals with the mutagenic effects of various chemicals, radiations and the consequences of human health exposure to such mutagens. Investigations on the mechanism of mutagenesis and application of test systems to characterize mutagens are the important goals of this specialized area. Significantly, it has provided the primary source of data on health effects for chemical safety evaluation on existing and new products, without which many potentially hazardous chemicals would have been identified only through human experience.

Broadly speaking, mutagenesis include induction of DNA damage and genetic alterations that range from one or few DNA base pairs to gross changes in chromosome structure or number. An agent that causes mutation is a mutagen and the more specialized term clastogen or aneugen is reserved for agent that causes chromosome aberration or aneuploidy respectively.

Cytogenetic studies further, offer a direct link between mutagenicity tests in experimental organisms leading to effects in humans (Bender et al.,1988; Sorsa et al., 1992). There are two major reasons of concern about human exposure to mutagens. An increase in mutation rate in human germ cells may cause an increased incidence of genetic diseases in future generation and second, mutations in somatic cells may contribute to various disorders and further, genetic alterations have already been implicated in carcinogenesis (Hoffman, 1994). A common chemical or physical agent can facilitate interaction with nucleic acids; the universality of target molecule is the key to the discipline of the genetic toxicology (Dolls, 1994).

It all began with the mutation research by Muller (1927) that X ray cause sex linked lethal mutations in fruitfly *Drosophila melanogaster.* The mutagenic properties of other chemicals were observed much later (Oehlkers, 1943; Auerbach and Robson, 1946). Subsequently, suitable routine protocols for detection and evaluation of environmental mutagens were and are still being

devised. Along the earliest attempts by Demerec (1956) and Hartman et al., (1971), the awareness that some of the hereditary diseases observed in human populations might be environmental in origin developed in due course (Rowley et al., 1983; Burgdorf et al., 1997). Increased attention has now been focussed on the potential genetic risks in animals and humans exposed to environmental chemicals. Over two hundred in vitro and in vivo tests have been developed and used to determine the intrinsic mutagenicity and potential carcinogenicity of chemicals (Waters et al., 1988). The short-term tests among others include, gene mutation in *Salmonella*, cultured mammalian cells gene mutation and chromosomal aberrations, and mammalian bone marrow cytogenetic methods comprising micronucleus and chromosomal aberrations. Thus various compounds were tested in both somatic and germ cell assays to discrimine mutagens and non mutagens. The data was compiled by the U.S Environmental Protection Agency and International Agency for Research on Cancer Generating Activity Profile (EPA; IARCGAP, available on MDW) as well as by EPA GENE-TOX data base (available on TOXNET).The quantitative results for various important chemicals are summarized in table.1A and B for ready reference.

The intensive use of chlorinated phenoxyalkanoic acids as herbicides in agriculture and forestry and also as defoliants made 2,4-Dichlorophenoxyacetic acid (2,4-D) as omnipresent. 2,4-D was the first successful selective herbicide developed. It was introduced in 1946 and rapidly became most widely used herbicide in the world and function as systemic herbicide. After 50 years of use, 2,4-D is still the third most widely used herbicide in the United States and Canada and is being widely distributed to farmers in India, while Pentachlorophenol (PCP), a chlorinated phenolic compound, is a man-made substance, not occur naturally, and until recently the most widely used biocide in USA as well as in our country.

A quick glance of Table 2 will show that the sale of herbicides exceeded the combined sales of insecticides and fungicides. A large amount of this steep rise was due to 2,4-D. However, the genetic effects of either chemicals have been

Table 1 A : Summary of the results of various types of test in some important organisms using various chemicals (Waters et al., 1994)

Chemicals	Germ cell mutation b								Bacterial mutation	Mammalian cells c		Bone marrow c	
	S L P	S L O	C C C C G E	C G G	O	D L M	D L R	M H T	ames test	CA	GM	CA	MN
Agents negative in multiple germ cell assays													
Dichlorvos	-	-	-			-			+	+	+	-	-
PCBs			-				-		-	?		-	-
Ethylene dibromide						-	-		+	+	+	-	-
Acrylonitrile			-			-			+	+	+	-	-
Cyclohexylamine			-	-		-			-	+	-	-	
									-				
Nitrofurantion	-					-			+	?			-
2,4-Dinitrotoluene						-	-		+	-	?		-
Formaldehyde	-					-			+	+	+	-	-d
Cyclamate, calcium e			-			-	-	-	-	+	-	-d	-
Caffeine	-		-			-			-	+	-	?	-
Parathion			-			-			-			-	
Theobromine			-	-					-	?	+	-d	+
Isoniazide	-	-	-	-		-			+	+		-d	
Cadmium			-			-	-		-	+	+	?	+
Arsenic +3			-			-			-	+	-	?	+
Benomy1				-			-		-	+	+	-d	+
Propylene oxide						-	-		+	+	+		+
Vinyl chloride						-	-		+		+	+	+
Methl parathion			-			-			+	+	+	+	+
Agents confirmed negative in a single germ cell assay													
Dieldrin						-			-		+	-	
1,1-Dimethylhydrazine						-			+		+		-
Epichlorohydrin						-			+	+	+	?	-
Chloradane						-			-		+	+	
Folpet							-		+	+	+		
Hexachlorobenene								-	-	-			

a Positive (+), negarive (-) and conflicting or inconclusive (?) test result; F=female treatments positive. b Germ cell tests include the mouse specific locus tests: SLP = post-spermatiogonial and SLO = other stages: chromosomal aberration in vivo: CCC = spermatocytes treated and observed, CGC = spermatogonia treated and spermatocytes observed, CGG = spermatogonia treated and observed, and COE = oocytes or embryos: dominate lethal tests: DLM = mice and DLR = rats; and the mouse heritable translocation test (MHT).

c Mammalian tests include chromosomal aberrartions (CA) gene mutation (GM) and micronuclei (MN).

d Test result corresponds to the same species tested for dominant lethals; formaldehyde, + in rats; Ca cyclamate, + in gerbils; isoniazide, - in rats; benomyl, - in mice.

e Sodium cyclamate was negative for DLM, CGG and CGC but caused chromosomal aberrations in somatic cells in vitro.

Table 1 B : Summary of the results of various types of test in some important organisms using various chemicals (Waters et al., 1994)

Chemicals	Germ cell mutation b									Bacterial mutation	Mammalian cells c		Bone marrow c	
	SLP	SLO	CCC	CGC	CGG	COE	DLM	DLR	MHT	ames test	CA	GM	CA	MN
Agents positive in multiple germ cell assays														
Cyclophosphamide	+		+	-	+	+	+	+	+		+	+	+	+
Myleran	+	-		-	+									
Ethylene oxide	-	+												
Methyl methanesulfonate	+	-	+	-		+								
Ethyl methanesulfonate	+	-												
Mitomycin C	-	+	+	-	+	+	+		+	+	+	+	+	+
Ethylnitrosourea	+	+		-	+		+		+	+	+	+	+	+
Methylnitrosourea	+						+		+	+	+	+	+	
Acrylamide	+	+	+	-	+		+	+	+	-	+	+	+	+
Triethylenemelamine	+	+	+	+	+		+	+	+	+	+		+	+
Procarbaine HCL	+	+			+		+		+	-		+	+	+
Hycanthone methanesulfonate	?	-		-		+	F	+		+		+	+	+
Dibromochloropropane	-	-			+		-	+		+	+		+	+d
Thiotepa			+		+	+	+		+	+	+	+	+	+
Triaiquone				-		+	+		+	+	+		+	+
Trimethyl phosphate							+		+	+			+	+
Isopropyl methanesulfonate				-		+	+		+			+	+	
Tepa							+		+	+	+		+	
6-Mercaptopurine	-	-	+				+		-	+	+	+		+
Cisplatin			+	?	+		F			+	+	+	+	
Bleomycin			+	+	+		F			-	+	+	+	?
Ethanol					-	+	+	+		-	-	-	-	-
Diethyl sulfate	+						+			+	+	+		
Agents confirmed positive in a single germ cell assay														
Adriamycin				+			F			+	+	+	+	+
Benzo (a) pyrene		-					+			+	+	+		+
Nitrogen mustard e							+			+	+	+		+
Methotrexate						+				-	+	+	+	+
Hexametapol							+			-	-	?	+	+
Methyl tepa							+			+				+
Methyl chloride							+			+		+		

a Positive (+), Negative (-), and conflicting or inconclusive (?) test result are indicated; F=female treatments positive.

b Germ cell tests include the mouse specific locus tests: SLP = post-spermatogonial and SLO=other stages; chromosomal aberrations in vivo: CCC=spermatocytes treated and observed, CGC=spermatogonia treated and spermatocytres observed, CGG = spermatogonia treated and observed and COE= oocytes or embryos; dominatnt lethal tests: DLM=mice and DLR=rats: and the mouse heritable translccation test (MHT)

c *Mammalian tests include chromosonal aberrations (CA) gene mutation (GM), and micronuclei (MN).*

d Positive in rats but negative in mice.

e Includes nitrogen mustard, nitrogen mustard N-oxide and chlormethine.

Table 2: Production and sales of 2, 4-D and other herbicides in comparison with other organic pesticides (Seiler, 1976)

Year	US production (tons)				Use (tons)		US sales (million dollars)		
	2, 4-D	Other herbicides	Insecticides	Synthetic organic pesticides	2, 4-D	Other herbicides	Herbicides	Insecticides	Fungicides
1945	450								
1950	5 600					640 a			
1960	15 500	15 200		296 000	2500	3950 a			
1964	23 800	42 800					200		
1965			206 000	400 000					
1967			215 000	480 000			450	301	55
1968	36 000	1 07 000	248 000	545 000		4500 a	505	335	60
1969	19 000	1 24 000	256 000	505 000			575	375	65
1970	20 000								
1974	27 000						1350	600	
1975					1600	3400 b			
1977	30 000								

a West Germany (FRG)

b Sweden.

less investigated. The available data on 2,4-D and PCP are reviewed and presented as a separate section in the ensuing text.

Review of literature on 2, 4-D

A brief resume on physical and chemical properties of 2,4-D has been presented in Box 1. Chlorophenoxyacetic herbicides mimic the action of indolacetic acid, a natural plant product hormone. First put to the use in agricultural practice in the mid 1940's, 2, 4-D, leads to differential growth rate in petioles, thicking of leaves, increase in turgor and drying of stem and leaves in treated plants (Crafts and Robins, 1973). The mode of action of such compounds has long been thought to involve some influence on the nucleic acid metabolism. In plants it has indeed been found that 2,4-D induced accumulation of DNA, RNA and protein (Mairer et al.,1971; Ashton et al., 1974; Corbett et al.,1974; Loos et al.,1975; Nagy et al.,1975; Wegler, 1977). Human exposure to such compound usually occurs through inhalation, skin contact or ingestion through accident or contaminated food. Occupational exposure of man to this compound was reported to cause asthenospermia, necrospermia and teratospermia (Lerda and Rizzi, 1991). The evidence of carcinogenicity of 2,4-D in animals and humans is indicated and therefore, genotoxicity has been tested in various organisms. Some of the findings are summarized in Table·3.

It is clear from the summarized data that although 2,4-D has been reasonable tested for mutagenicity but found to be non mutagenic in most systems and that not many reports have appeared in mice; the matter with respect to mutagenicity is yet not resolved at least in mice, In one case significant increase of damage occurred in chromosomes in cultured human cells at low exposure levels (Schlop et al., 1990) and yet 2,4-D reportedly not damaged DNA in human lung cells (Seiler, 1978). The evidence is too equivocal to draw any conclusions.

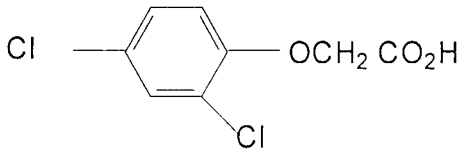
There is also some questions about whether the tumors are associated with a specific organ or are non-specific. Female mice given a single injection of 2,4-D developed cancer, reticulum-cells sarcomas (Forest Service, 1984).

The Pesticide Manual

Incorporating The Agrochemicals Handbook
TENTH EDITION
Edition, Clive Tomlin

2,4-D — Herbicide

aryloxyalkanoic acid



NOMENCLATURE
2, 4-D
Common name 2, 4–D (BSI, E-ISO, (m) F-ISO, WSSA), 2, 4-1 A (JMAF).
IUPAC name (2, 4-dichlorophenoxy) acetic acid.
C.A. name (2,4-dichlorophenoxy) acetic acid. CAS RN [94-75-7].

2,4-D-butotyl CAS RN [1929-73-3].
2,4-D-butyl CAS RN [94-84-4].
2,4-D-dimethylammonium CAS RN [2008-39-1].
2,4-D-diolamine CAS RN [5742-19-8].
2,4-D-isocyl CAS RN [25168-26-7] (formerly [1280-20-2]).
2,4-D-isopropyl CAS RN [94-11-1].
2,4-D-rolamine CAS RN [2569-01-9].

PHYSIOCO-CHEMICAL PROPERTIES
2,4-D
Mol. Wt. 221.0 Mol. Formula $C_8C_6Cl_2O_3$
Form Colourless powder. M.p. 140.5 °C V.p. 1.1 x 10^{-2} Pa (20 °C) SG/density 1.565 (30 °C) K logP =2.58-2.83 (pH 1) Solubility In water 311 mg / l (pH 1,25 °C). In ethanol 1250, diethyl ether 243, heptane 1.1, toluene 6.7, xylene 5.8 (all in g / kg, 20 °C). Insoluble in petroleum oils. Mono-n-butylamine salt : In water at 30 °C,18 g/l. Stability 2,4-D is a strong acid, and forms water-soluble salts with alkali metals and amines. Hard water leads to precipitation of the calcium and magnesium salts, but a sequestering agent is included in formulatons to prevent this. PKa 2.64.

2,4-D-butotyl
Mol. Wt. 321.2 Mol. Formula $C_{14}H_{18}Cl_2O_4$.

2,4-D-butyl
Mol. Wt. 277.1 Mol formula $C_{12}H_{14}Cl_2O_3$.

2,4-D-dimethylammonium
Mol. Wt. 2661.1 Mol. Formula $C_{10}H_{13}Cl_2NO_3$
M.P. 85-87 °C Solubility In water at 20 °C, 3 kg/l. Soluble in alcohols an acetone.
Insoluble in kerosene and diesel oil.
2,4-D-Diolamine
Mol. Wt. 326.2 Mol. Formula $C_{12}H_{17}Cl_2NO_5$.
s
2,4-D-isocytyl
Mol. Wt. 333.3 Mol. Formula $C_{16}H_{22}Cl_2O_3$.

2,4-D-isopropyl
Forms Colourless liquid. M.p. 5-10 °C and 20-25 °C (two forms) B.p. 130 °C/l mm Hg Solubility Practically insoluble in water; soluble in alcohols, most oils.

2,4-D-sodium
Solubility In water 18 g/l (20 °C).

2,4-D-trolamine
Mol. Wt. 370.2 Mol. Formula $C_{14}H_{21}Cl_2NO_6$
M.p. 142-144 °C Solubility In water 4.4 kg/l (30 °C).

COMMERCIALISATION
History The potent effects of its salts on plant growth were first described by P.W. Zimmeman & A. E. Hitchock (Contrib. Boyce Tompson Inst., 1942. 12.321) and the early history is covered in The Hormone Weedkillers; C. Kirby (1980).

Table 3: Summary of results on the genotoxicity of 2,4-D in different test system (Tripathy et at., 1993)
(w, weak; + positive; – negative)

Test organism	Test Type	Conclusion
Salmonella typhimurium	Mutation (histidine reversion assay)	w
		–
Bacillus subtilis	Mutation	+
E. coli	Mutation	–
Saccharomyces cerevisiae	Mitotic recombination	+
		–
Aspergillus nidulans	Mitotic recombination	+
Allium cepa	Clastogenicity, spindle dysfunction	+
Allium ascalonicum	Clastogenicity, spindle dysfunction	+
Orya sativa	Morphological mutants	+
Wheat	Mutation	+
	Clastogenicity, spindle dysfunction	
	Micronuclei	+
Barley	Karyological abnormality	+
Drosophila melanogaster	Sex-linked recessive lethal test	–
		+
	Non-disjunction	–
	Wing spot test	+
Rats & mice	Clastogenicity, polyploidy	+
Chinese hamster V79 fibroblasts	HGPRT mutation	+
Human SV-40 transformed cells	Unscheduled DNA synthesis	+
Human lymphocytes	Chromosome aberrations,	+
	Sister-chromatid exchange	

However the above studies on 2,4-D related carcinogenicity were considered inadequate by International Agency for Research on Cancer (IARC, 1974). In humans, a variety of studies gave conflicting results, though some studies suggested an association of 2,4-D exposure with cancer (Hoar et al.,1986; Zahm et al., 1990). An increased occurrence of Non Hodgkins lyphomas was common among a Kansas and Nebraska farm population associated with the spraying of 2,4-D. Other studies from New Zealand, Australia and Vietnam were negative (Fedral Register, 1990). There remains considerable controversy about the methods used in these studies and their results.

Since, 2,4-D increasingly used in water bodies to kill weeds, studies on aquatic organisms like fish were natural. Some formulations of 2,4-D were highly toxic to fish while others were less so (National Research Council, Canada; 1978). Chromosomal alterations have been studied for many decades and it was immediately appreciated that they are associated with malignancy (Boveri, 1902). Some deletions have a break point in a similar location to constitutional fragile sites in response to chemical carcinogens (Whang-peng, 1982). Furthermore, mutagenic chemicals have the probability of inducing carcinogenic effects and majority of these chemicals have been shown to cause tumors at specific or multiple sites in fish (Harshbarger and Clark, 1990). A clear association between chromosome rearrangements and cancer has been found and virtually all tumors contain structural as well as numerical chromosome rearrangements (Solomom et al., 1991; Rabbits, 1994; Mitelman, 1994). Fish responds analogous to mammalian test species to chemicals by inducing peroxisome proliferation (Yang et al., 1990) and cytochrome P450 monoxygenases to metabolise many carcinogens in liver (Stegeman and Lech, 1991). In a comparative study, Regal et al (1983) reported that fish tends to be sensitive to UV radiation and mutagenic carcinogens than mammalian cells. These observations were based on the cytogenetic end points in toxicological evaluation of industrial chemicals, pesticides and development of new pharmaceuticals and therapeutic compounds.

That the fish can serve as an excellent model for monitoring genotoxic

effects by measuring the chromosomal aberration and micronucleus test and the utility of these tests including apoptotic studies on 2,4-D in various fish has been amply shown by many studies (Ali and Ahmad, 1997; Ahmad et al., 2001; Farha et at., 2001). Thus the quantitative risk from exposure of fish to chromosomes damaging agents 2,4-D in this case, is a useful addition to the screening test designed to protect public health.

As a technical advancement, many protocols were developed to facilitate chromosome preparation during the past several years. Hsu (1952) introduced, the use of hypotonic treatment for accurate analysis of human chromosomes, which is considered as boon for all karyological studies. A revolution in chromosome cytology took place when pretreatment with colchicine coupled with squashing was introduced (Roberts, 1964), followed by the flame drying method (Denton and Howell, 1969). The limiting factor apparently is obtaining consistently good chromosome spread from the tissue and for that, many different techniques are being used to perform karyological studies, either in vivo or in vitro. Such methods are based on the use of colchicine to quickly block the proliferating cell population from suitable organs like kidney, spleen, gill epithelia and other tissues (Ohono et al., 1965).

Bacterial tests so far have had a negative record with phenoxyacids. Fiscor et al., (1972) tested 2,4-D dimethylamine salts as commercial formulation in five strains of *E. coli*. Andersen et al., (1972) used *Salmonella* strains for their investigation on many herbicides, like 2,4-D; 3,4-D; 2,4,-DB; 3,4-DB; 2,4-DEP; 2,4-DP and MCPB. They also investigated 2,4-D and 2,4-DEP in forward and reverse mutation experiments on phage T_4. The Ames test of *salmonella* mutants by Carere (1976) who tested mecoprop in the four strains as well as forward mutational system in *Streptomyces coelicolor*, no mutagenic activity was found. Beside these studies, negative results in bacterial assay are also on record (Fahrig, 1974; Wild, 1975). There are however, a few reports on marginal effects of phenoxyacids in bacterial systems, which may indicate a certain weak potential for DNA damage (Buselmaier, 1972; Zetterberg, 1977).

On the other hand, cytogenetic studies on phenoxyacids in plant cell culture are manifold and the results can broadly be divided in two groups. In the first group, no consistent trend was obvious regarding the production of chromosomal aberrations or other mitotic irregularities under the influence of 2,4-D and even negative association of anaphase irregularities are described (Singh et al., 1975; 1977). The second series of investigation however, showed positive co-relation of polyploidy and chromosomal aberrations with varying concentrations of 2,4-D (Kallak et al., 1971; Dev et al., 1973; Libbenga et al., 1973; Butcher et al., 1975). Earlier, Derscheid (1952) observed morphological abnormalities in barley, which were not however transmitted to succeeding generations. The induction of heritable changes by 2,4-D in oats though confirmed later (Seiler 1978). An analogous observations on somatic mutations in *Pelagonium zonale* by the same compound produced a slight but significant increase in mutational frequency (Pohlheim et al., 1977). Chromosomal aberrations were also noted in pollen mother cells of *Vicia faba* in rather higher frequencies after spray treatment of the plants or soaking of seeds with 2,4-D solutions (Amer et al., 1974).

In whole plants, some authors described pronounced effects on chromosomes and only rarely no influence on chromosomal aberrations (McMohan et al., 1960). The common cytological observations included polyploidy (Kar, 1975), stickiness, lagging chromosomes, micronuclei and chromosomal fragmentation (Grant et al., 1973). At high doses 2,4–D produce chromatid breaks in root cells of *Allium cepa*, whereas in longer treatment in all concentrations chromosome breaks were observed (Croker et al., 1953). A very interesting result was cited with 2,4-D in this organism where C tumors were conspicuous (Bushra et al; 2001). Under normal field conditions several weeds respond to 2,4-D treatment by aberrant mitosis (Tomkins et al., 1976). C-mitosis and endo polyploidy in 2,4-D treated plant cells has been hypothesized as being due to an interaction of 2,4-D with spindle fibre protein (Muhling, 1960).

Investigations in *Drosophila* with phenoxyacids pointed a mutagenic potential, thus showing somatic mutations, chromosome loss and other gross

effects and sex lethality (Tripathy et al., 1993). A sex linked, genetically unstable test system using somatic mutation for altered eye color was used to test the mutagenic activity of 2,4-D in fruit fly and the results were compared with those from an analogous but genetically stale and less sensitive system (Rasmusson et al.,1977). 2,4-D was also found to be slightly mutagenic for *Drosophila* in recessive lethal test. Sex linked recessive lethality is known to be most sensitive technique in mutation detection with *Drosophila* (Vogel et al., 1974).

Review of literature on PCP:

PCP and other chlorophenols are used primarily for protecting wood from fungal growth. Other applications include; indoor disinfectant, leather and textile and herbicide use. Although in some countries their use has been discontinued or abandoned as a result of severe restrictions, PCP is still an important pesticide in many developing countries including India more so because of its low cost and broad spectrum use. In some developed countries like France and USA, several thousand tons of PCP are still being produced annually (IARC, 1991; Mc Connell et al., 1991). Even where PCP use has been abandoned, it continues to be an important environmental contaminant, since it is imported via various materials treated with it.

PCP can be metabolized by many aquatic and soil micro organisms, though environmental conditions are usually unfavorable for its bio-degradation (Orser and Lange, 1994; Laine and Jorgensen, 1996). Slow elimination in surface waters, high persistence in sediments, formation of stable metabolites, and the limited adaptation of micro organisms to chlorophenols owing to their high microbial toxicity imply that chlorophenols are practically non-biodegradable in the aquatic environment (UBA, 1996). Importantly, its trace contaminants, polychlorinated dibenzo-p-dioxins (PCDDs) and poly chlorinated dibenzofurans (PCDFs) are not metabolized and hence hazardous (Bajpai and Banerji, 1992; Sal Kinoja et al., 1989).

Chlorophenols are relatively water soluble in the anionic form therefore, soil contamination may lead to the contamination of ground water as well. The

solubility of the dioxin/furan contaminants is very low and these contaminants are readily absorbed onto soil particles and other surfaces; hence, the risk of contamination of finished drinking water is not great. In surface waters however, organic or clay particles easily transport dioxins/furans to distant sites from their origin (Koistinen et al., 1995). Significantly, PCP is accumulated by aquatic organisms through uptake from the surrounding water or along the food chain (WHO, 1987). Other salient features about its physical and chemical properties have been presented in Box 2.

The kinetics of PCP has been reviewed on a number of occasions (Ahlborg and Thunberg, 1980; WHO, 1987; IARC, 1991). A general overview and recent results are presented below, in reference to PCP.

PCP is well absorbed orally (Reigner et al., 1992), although bioavailability may be influenced by food (Yuan et al., 1994). During occupational exposure, PCP is well absorbed through dermal and pulmonary routes (IARC, 1991) which is well supported by animal studies (WHO, 1987; Regner et al., 1992). It is likely that PCP is conjugated to glucuronic acid and excreted into urine, even if the rate of glucuronidation is controversial (Reigner et al., 1992; Wester et. al., 1993; Yuan et. al., 1994). There is some uncertainty as to the elimination rate of PCP (Reigner et al.,1992)

A number of toxic effects in short-term tests have been attributed to impurities present in technical grade PCP preparations. Rats receiving technical grade for long periods had slow growth rates, liver enlargement, porphyria, and increased activities of some liver microsomal enzymes (Goldstein et al., 1977; Kimbrough and Linder, 1978). This was not so in purified PCP (Pohjanvirta and Tuomisto, 1994). Some effects on enzyme activity, such as strong inhibition of sulfotransferase activity are due to PCP itself (Boberg et al., 1983).

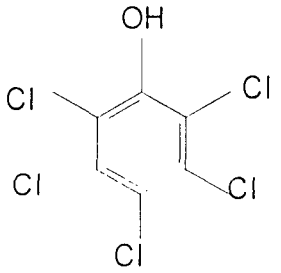
A number of effects on immune systems have been also been noted: for example reduced humoral immunity and impairment of T-cell cytolytic activity and decreased cell-mediated and humoral immunity in mice and rats are well studied (Kerkvliet et al., 1982; Exon and Koller, 1983). Although not teratogenic, PCP is embryo/fetotoxic at high doses in rats (Schwetz et al,1978; Exon and Koller,

The Pesticides Manual

Incorporating The Agrochemicals Handbook
TENTH EDITION
Editon Clive Tomlin

pentachlorophenol

Insecticide Fungicide
Herbicide



NOMENCLATURE
Pentachlorophenol
Common name PCP (WSSA, JMAF), pentachlorophenol (BSI, E-ISO, F-ISO, accepted in lieu of a common name)
780 pentachlorophenol

IUPAC name pentachlorophenol,
C.A. name pentachlorophenol. CAS RN /87-86-5/ Other names PCP.

Sodium pentachlorophenoxide
Common name sodium pentachlorophenoxide (BSI, E-ISO), pentachlorophenate de sodium(F-ISO).
IUPAC **name** sodium pentachlorophenoxide : sodium pentachlorophenate.

PHYSICO-CHEMICAL PROPERTIES
Pentachlorophenol
Mol. Wt. 266.3 Mol. Formula C_6HCl_5O
Form Colourless crystals, with a phenolic odour, (tech., dark grey), M.p. 191 °C; (tech. 187-189 °C) B.p 309-310 °C (decomp.) V.p. 16 Pa(100 °C). SG/density 1.98 (22 °C) Solubility In water 80 mg/l (30 °C). Soluble in most organic solvents, e.g. acetone 215 g/l(20 °C). Slightly soluble in carbon tetrachloride and prafins. The sodium, calcium and magnesium salts are soluble in water. Stability Relatively stable and non-hygroscopic. PKa it is a weak acid, pK, 4.71 Flash point Not flammable

Pentachlorophenyl laurate
Mol. Wt. 448.6 Mol. Formula $C_{18}H_{23}Cl_5O_2$.

Sodium pentachlorophenoxide
Mol. Wt. 288.3 Mol. Formula C_6Cl_5NaO
Solubility Crystallises from as a monohydrate, solubility in water 330 g/l (25 °C).
Insoluble in petroleum oils.

COMMERCIALIZATION
History Introduced c. 1936 as a timber preservative and later used as a general disinfectant. Manufacturer Chapman.

APPLICATIONS
Uses Pentachlorophenol is used to control termites and, frequently, as an ester (such as pentachlorophenyl laurate) to protect wood from fungal rots and wood-boring insects, and as a general herbicide. The sodium salt is used as a general sisinfectant, e.g. for trays in mushroom houses. Formulation type GR; WP; Ol.

ANALYSIS
Product analysis by titration with alkali (MAFF Ref. Bk., 1958, No. 1, p. 64), Residues determined by colorimetry of derivatives (W.W. Kilgore & K.W. Chang Pestic, Plant Growth Regul, Food Addit., 1967, 5, 313; Anal Methods Pestic. Plant Growth Regul., 1972, 6, 581) or by gic (AOAC Methods, 1990, 985. 24)

MAMMALIAN TOXOCOLOGY
Pentachlorophenol
Reviews Environment Health Criteria 71 (WHO, 1987). Acute oral LD_{50} for rats 210mg/kg. Skin and eye Acute percutaneous LD_{50} not available Irritating to skin (the solid and aqueous soloution> 10 g/1), eyes and mucous membranes. NOEL No deaths occurred among dogs and rats receiving 3.9-10 mg daily for 70-190,d. Toxicity class WHO Ib; EPA II.

1982; Welsh et al., 1987). The high dose found to reduce the number of offspring, neonatal body weight, neonatal survival and growth of weanlings (Schwetz et al 1978). Individual developmental effects of purified (98%) and technical grade (88.4%) of PCP when investigated, it was noted that purified PCP induced delayed fetal development without simultaneous material toxicity, while technical grade showed no such effects in rats (Schwetz et al., 1974).

It was Seiler (1991) who studied the genetic toxicology of PCP thoroughly. Apart from few scattered observations on mutagenic activity attributed to oxygen radical formation by its metabolite, PCP apparently produced no DNA damage. The subsequent studies supported these findings (Jansson and Jansson, 1992; Dahlhaus et al., 1994, 1995; Naito et al., 1994; Waidyanath et al., 1994; Sai-Kato et al., 1995; Wang and Lin, 1995). There is some evidence of weak clastogenic effects in chromosomal aberration assays in vitro and in lymphocytes of exposed persons in vivo (Bauchinger et al., 1982; Seiler, 1991). 2,4,6-trichlorophenol reported to induce chromosome mal-segregation rather than mutations in CHO cells (Jansson and Jansson, 1992; Armstrong et al., 1993). Early carcinogenicity studies on PCP were negative or equivocal (Innes et al., 1969; Schwetz et al., 1978), but there were deficiencies in these studies (IARC, 1991). Dose-dependent and significantly elevated levels of hepatocellular adenomas and carcinomas, phaeochromocytomas, and haemangiosarcomas were observed in mice. Overall, the purer preparations were also not less tumorigenic than the technical-grade PCP. Hepatic tumours and phaeochromocytomas were more common in males, whereas haemangiosarcomas were seen only in females. McConnell et al., (1991) concluded that tumours caused primarily by PCP itself and not by impurities. There is a lack of carcinogenicity data on 2,3,4,6-tetrachlorophenol. 2,4,6-trichlorophenol although indicated hepatocellular carcinomas or adenomas in mice of both sexes and lymphomas and leukaemias in male rats (National Cancer Institute, 1979). However, generally studies so far provide sufficient evidence for PCP carcinogenicity in experimental animals (IARC, 1982).

The paramount difficulty in interpreting human studies, especially by long

term observations has been simultaneous exposure to other chemicals (Johnson, 1990; Saracci et al., 1991). Those working in chemical industries are often exposed simultaneously to chlorophenols, dibenzodioxins, dibenzofurans, and the chemical being synthesized. Similarly those working in forestry or agriculture are often exposed to chlorophenols, chlorophenoxy acids, dibenzodioxins and dibenzofurans as well as other pesticides.

PCP and other higher chlorinated phenols have been shown to act cellularly to uncouple oxidative phosphorylation and to inhibit ATPase and several other enzymes (Jorens and Schepens, 1993). This lead to excessive heat production and fever. Symptoms of acute poisoning include central nervous system disorders, dyspnoea, and hyperpyrexia and cardiac arrest along with rigor mortis (IARC, 1991). Acute human poisoning is seen only after large accidental or suicidal doses.

The human toxicology of PCP was reviewed by Jorens and Schepens (1993). In addition to metabolic, respiratory, and central nervous system effects, the clinical features include effects on skin and haematopoietic tissue, renal and gastrointestinal deficiencies. These effects have occasionally been seen after heavy occupational exposure. For example in a Chinese PCP production plant, high prevalence of chloracne, increased urinary porphyrin excretion, and decreased motor nerve conduction velocities were observed in the high-exposure area (Cheng et al., 1993; Coenraads et al., 1994). Drinking-water exposure was associated with gastrointestinal symptoms (nausea, pains, diarrhoea) and mild skin disorders like itching and eczema (Lampi et al., 1993); some of these can assumed to be due to chlorophenols as no dioxin exposure was noted (Vartiainen et al., 1995).

Immunological abnormalities were also observed in one of the study where T-cell activation and autoimmunity, functional immunodepression, and B-cell dysregulation were noted (McConnachie and Zahalsky, 1991). Colosio et al (1993) found no major clinical or laboratory signs of immune deficiency, while a study implicated chlorophenols, chlorophenoxy acids, chlorinated dibenzo-p-dioxins, and chlorinated dibenzofurans in the causation of cancer, especially soft-

tissue sarcoma and non-Hodgkin lymphoma (Lilienfeld and Gallo, 1989; Johnson, 1990; IARC, 1991). Owing to several inconsistencies, the case remained debatable. Generally, the role of chlorophenols remain more elusive than that of other chemicals of the group because the exposed groups were smaller and the number of cases relatively less.

There is some, although not irrefutable, evidence that chlorophenol preparations, including PCP, may cause cancer in humans. There seems to be no reason to believe that the problem could be overcome by advocating more purified preparations. In fact, there is little evidence that minor carcinogenic impurities, such as Trichlorinated di-bezo-P-Dioxins (TCDD) and other chlorinated dioxins and furans, would be more important than the main chemical, and there is a limited direct evidence on the basis of both animal experiments and epidemiological studies, that pure chlorophenols may, at relevant concentrations pose a risk of cancer in humans. As far as water is concerned, only the risk of chlorophenols appear reasonable, as the impurities are practically non-soluble in drinking water.

Thus in view of the conflicting reports and inconclusive results discussed at length, the evidence of carcinogenicity of 2,4-D and PCP in animals and humans was judged inadequate by competent organization (IARC, 1986). However, the reproductive effect of these compounds has been able to inflict the reproductive structural changes at moderate doses in animals. This vindicate that the humans may be at risk with the exposure of these compound, even if no direct evidence of reproductive problems associated with them exist. Besides, there remains considerable controversy about the methods used in various studies and their results. The present experiments are therefore, designed and persued to answer some of the above malaise in accordance with the laboratory practice regulations and applicable toxicology guidelines (MAFF,1985; EPAPAG, 1984;EEC, 1987).

The proposed work emphasize the specific objective: Assessing the cytogenetic damage induced by 2,4-D and PCP in the mouse bone marrow cells using in vivo assay by cytogenetic parameters such as chromosomal test (CA),

micronucleus assay (MNT) and the mitotic indices profiles (MI). The out come of these studies are compared to previously published in vivo genotoxic assays at appropriate places.

CHAPTER

Materials and Methods
Chromosomal Aberration Test (CA)
Micronucleus Test (MNT)
Mitotic Index (M.I)

MATERIALS AND METHODS.

The protocols and related aspects of these experiments strictly followed the laid down norms for genotoxicity testing (MAFF,1985; EPAPAG, 1984; EEC, 1987; 1988; EPAFI, 1990; EPEPAG, 1991). A brief detail of each event is described below:

A. Test Animal

Mus musculus, 200 in numbers comprising both the sexes were procured from CDRI Bangalore. They were of 10-18 week old with an average weight of 26.21±12-25 gm, and housed in different groups in different cages maintained under standard conditions of temperature, humidity and light. The animals were given standard food and water ad libitum. *Mus musculus* has a standard karyotype of 20 pairs of acrocentric chromosomes including 19 pairs of autosomes and a pair of distinct X and Y sex chromosome.

B. Cytogenetic Testing Parameters :

(i) Chromosomal Aberration test (CA):

The CA was used for the detection of structural chromosomal aberrations induced by test compounds.The chemical tested was administered intraperitonealy. The somatic tissue used was bone marrow. Classical protocol using bone marrow from rodents recommended by ad-hoc committee of the Environmental Mutagen Society and the Institute of Medical Research (1972) was adopted. The extent and the type of aberration was carefully studied. Structural chromosomal aberrations classified in two types; chromosome or chromatid. An increase in polyploidy was also oberved as it may indicate that a chemical has the potential to induce numerical aberrations. Since the majority of chemical mutagens induced aberrations are of the chromatid–type, chromosome-type aberrations also occur and therefore considered in the present study.

(ii) Micronucleus Test (MNT):

Micronuclei are cytoplasmic chromatin containing bodies formed when

accentric chromosome fragments or chromosome lag behind during anaphase and fail to become incorporated into daughter nuclei during cell division. Since genetic damage result in chromosome breaks, such structurally abnormal chromosomes, or spindle abnormalities lead to micronucleus formation. The incidence of micronucleus thus serves as an index of chromosome damage. Enumerations of micronuclei is much faster and less technically demanding than the scoring of chromosomal aberration.

(iii) Mitotic Index (MI):

The calculation of the mitotic Index usually based on the formula:

MI = Total no. of dividing cells $\times 100$ / Total no of cells observed

In such calculations interphase, pro metaphase and sub stages are not counted.

This approach is essential, but it does not often give the true index of mitotic division, thus mitotic index should be assessed at metaphase-anaphase stage because these stages actually entitle a cell to have processed the mitotic cycle. This is designated as active mitotic index and computed by:

$$Ami = (M+A) \times 100 / TC$$

Where, M, A and TC respectively stands for metaphase, anaphase, and total number of cells observed. MI was observed by this formula and any inhibition of mitotic indices was taken as the cytotoxicity of the chemical.

Comparison on the basis concentration or duration of the test chemical was noted and contrasted with the controls to realize which agent effects more seriously over the other.

C. Test Chemicals:

The powdered form of Pentachlorophenol (99.9%) was obtained from Fluka Chemika Switzerland 76470/CH9470, CAS R No 87-86-5 while 2,4-Dichlorophenoxyacetic acid also a powdered form (98%) was purchased from the

Loba Chemie India Art.3300 under CAS No 94-75-7. Further details regarding physical and chemical features of either chemical are presented in Box 1 and 2 in Chapter I (Introduction and Review of literature).

D. Other Chemicals, Preparations of Solutions, Concentrations.

(i)Chemical

Colchicine (Loba Chemie) 25mg/50ml of distilled water. (injected @ 4mg/1000gm of body weight)

Hypotonic, KCL 0.075M 0.56 % (Ranbaxy Laboratories).

Cornoys fixative (methanol: glacial acetic, (3: 1).

Giemsa stain (5%) (Loba Chemie).

May- Gruenwald stain (Merck limited).

Sorensens buffer (pH-6.8)

Solution A, $Na_2HPO_4.2H_2O$ 5.938gm/100ml

Solution B, KH_2PO_4 4.539gm/100ml

25 ml of solution A + 25 ml of solution B added to make 50ml of solution

Xylene (s d Fine Chem limited).

DPX (Glaxo Laboratories)

Fetal Bovine Serum 5% (Sigma Laboratories)

Cyclophosphamide (Sigma Laboratories) 40mg/ 1000 (injected @ 40mg/1000gm of body weight.)

ii. Stock solutions

Pentachlorophenol (PCP) stock solution :
1.33mg /1ml of distilled water

2,4-Dichlorophenoxyacetic acid (2,4-D) stock solution:
4mg/1ml of distilled water.

iii Concentrations

PCP; 5, 15 and 20mg/1000gm of body weight.
LD 50 (male) 61mg/1000gm of body weight
LD 50 (female) 59mg/1000gm of body weight
(Seiler,1990)

2,4-D; 25, 50 and 75mg/1000gm of body weight.
LD 50 (male) 125mg/1000gm of body weight
LD 50 (female) 125mg/1000gm of body weight
(NIOSH ,1989)

Details regarding preparation of various concentrations of chemicals has been given below:

Amount of Solute	Ethanol	Distilled Water	Stock	In 1 ml Stock
PCP 20mg	1 ml	14 ml	15ml	1.33mg
2,4-D 40mg	-	10 ml	10 ml	4 mg
Solvent Control	1 ml	14ml	15ml	0.71ml

iv Duration 12, 24, 48 and 96h

v Type of treatment Intraperitoneal (i.p. injection)

E. Experimental Design

A lot of 120 mice were divided into four experimental groups. Exposed groups consisted of 72 mice in all, 36 each for PCP and 2,4-D. Twelve mice were used for each concentration @ 3 mice per duration in each chemical.

The control groups consisted of normal control (tap water), solvent control (1ml ethanol/100ml DW) for PCP and simple distilled water for 2,4-D, and a separate positive control, 40mg cyclophosphamide/1000gm of body weight. Each control group included 12 mice to a total of 48 mice in all.

At the end of the specific interval, 3 mice per duration were sacrificed by cervical dislocation and immediately dissected to obtain bone marrow for various cytogenetic preparations. The explained design was followed in CA, MNT and MI studies

F. Cytogenetic Procedures:

(i) Chromosome preparation from mouse bone marrow metaphase (Tijo and Whang, 1962).

The animals were injected intraperitoneally (i.p.) with a colchicine solution (4mg/1000gm) 2h prior to tissue sampling.

They were sacrificed by cervical dislocation. Both the femurs were extracted and cleaned from muscular tissue.

The bone was cut open at distal ends, the needle was inserted with mounted syringe and the bone marrow was flushed out using 5ml of pre-incubated KCl in a centrifuge tube.

This solution was properly homogenized and kept in a water bath at 38° C for 20 min. To this 1ml of fixative was added drop by drop and the solution was left at room temperature for 20 min.

It was centrifuged for 5 min at 100g. The supernatant was discarded and the pellet was saved. To this pellet, chilled fixative was added and centrifuged again. The pellet was saved again and supernatant was discarded. The process was repeated thrice and the final pellet was saved in 2ml of chilled fixative.

The slides were pre washed with alcohol and chilled in 70 % ethanol at 0°C. The cell suspension so was dropped onto the slides from a appropriate distance. The slides then passed over the flame to burn off the fixative immediately, air dried and stained for 5 min in Giemsa (5%) in Sorensens buffer (pH 6.8).

Satisfactory slides having well separated and extended chromosomes were mounted in DPX and randomanized.

All slides including controls were coded and scored blindly by a single observer. The slides were screened for analyzable metaphases, initially under low power (45X) and subsequently at high magnification (100X,oil)

Analyzable mitotic cells were selected on the criteria: well spread metaphases with 2n=40, or more complete number of centromere, fully aligned chromatid, no centromere splitting, no extensive overlap of chromosomes and good fixation and staining.

Chromosomal aberrations were classified as per nomenclature (Adler, 1980). The structural type of aberration was studied under two-sub types, chromatid and chromosome. Chromatid type aberrations involved one chromatid comprising chromatid gap (G), where an achromatic lesion was found with smaller than the width of one chromatid; chromatid breaks (B) having the true discontinuities with clearly dislocated fragment and translocations (T), where the fragments almost remained associated with chromosome of origin.

Chromosomal type of aberration, involved both the chromatids of a chromosome at identical sites or loci and comprised of abnormalities like gap (G') an achromatic lesion, having non-staining region not greater than the diameter of the chromatid; break or (terminal deletion) that involved only one chromosome; chromosomal exchanges, where two or more lesions in the same or different chromosomes were observed. On the other hand, multiple aberrations (MA) comprised of rings, dicentrics and aneuploidy, where the chromosome number of a cell deviated from a multiple haploid set, 20 in the present case.

Other abnormalities like improper spreading and clumping of chromosomes were included in stickiness and pulverization, both scored separately.

(ii) Micronucleus preparation from mouse bone marrow (Schmid, 1973)

Both the femurs were extracted from the test animals and muscular tissue cleared. The bone was cut open and the bone marrow was flushed out with 5% of foetal calf serum and the solution was homogenized properly. No hypotonic

treatment was given.

The solution was kept for 5 min at room temperature and the supernatant was discarded and pellet saved. The sediment was mixed with a pipette and a small drop of cell suspension was dropped at the one end of the clean dry glass slide and evenly smeared and air dried without fixation.

The staining procedure followed a combination of May-Gruenwald and Giemsa staining in succession: the slides were first covered with undiluted May-Gruenwald solution for 3 min and replaced by diluted (1:1) solution of the same stain with distilled water for 2 min followed by staining in Giemsa for 5 min. The slides were rinsed and dried, treated with xylene and mounted in DPX.

All slides including controls were coded and scored blindly by a single observer and screened for analyzable micronucleus under the same magnification as described earlier. In well stained slides, the nuclear material was deep purple in color while the cytosplasm of nucleated cells appeared as light blue. Normochromatic erythrocyte were orange yellow in color in contrast with polychromatic erythrocytes which were pink to purple. The differential staining thus allowed the clear discrimination between polychromatic erythrocytes (PCE's) and normochromatic erythrocytes (NCE's). The right area was chosen for scoring where the erythrocytes were well seperated, not folded, and clearly contoured.

(iii) Calculation of mitotic Indices (Hedge et al., 1995)

The mitotic index (MI) was calculated from a total of 2000 cells scored in each concentration category. Details regarding mathematical procedure and justification presented elsewhere in this chapter, (section B, iii).

G. Statistical Analysis:

All the scorings were done from slides under code. The percentage frequencies of chromosomal aberrations micronuclei and mitotic indices were calculated and the mean percentage and standard error for required groups computed.The data were admitted to the equality of proportion test (normal test) to find the significance of treated values over control by

$$Z = \frac{(p1 - p2)}{P(1 - P)\ (1/N1 + 1/N2)}$$

Where p1 and p2 are the frequencies of anomalies of the cells in control and treated; N1 and N2 are the number of cells studied in control and treated series and P = (p1+p2)(N1+N2). The results were considered significant at 5% level when $Z \geq 1.96$.

CHAPTER

Results
Discussion
Summary
References

RESULTS

Mutagenic effects of PCP and 2,4-D based on various parameters have been summarized in Tables 4 to 9. Dose and duration related trends of all the cytogenetic parameters have been represented graphically from Figures 1 to 8. The detailed results are discussed below.

Chromosomal aberrations, with three concentrations of Pentachlorophenol indicate that there was a clearly significant increase in aberration at 24 h interval though rising trend was evident in every concentration (Table 4); the fact also depicted in Figure 1. There was a definite decrease beyond 48 h. Table 5 show that there was a time dependent increase in C1 in 2,4-D, while concentrations C2 and C3 indicated that the injurious effect to the chromosomes was minimized at higher concentrations as durations progressed beyond 48 h (Figure 3). The effect clearly decreased with increase of time. In both test chemicals no significant increase was observed between normal and solvent control, however when contrasted with solvent control the rise was significant.

Both chemicals were able to indicate chromatid and chromosome type of aberrations, and the frequency of chromatid type of aberration was high as compared to chromosome. Also evident from statistical data was the chromatid type gaps were consistently and more frequently observed in all replicates of 2,4-D and PCP. Aberrations such as breaks, translocations, stickiness and pulverisations were also observed; a dose dependent increase in total aberrations were noted. A closer look of Figures 2 and 4 relating to PCP and 2,4-D showed an increase in stickiness and pulverisation from 12 to 24 h of treatment; followed by a decline as the time elapsed from 48 h onwards. No significant differences were observed in normal and solvent control for this class either. A maximum of 18.33 ± 0.05 frequency of aberration was recorded in PCP treated group at 24 h of C3 as compared to 15.66 ± 0.05 of 2,4-D, showing comparative effectiveness of the former. By and large, results for CA reveal that there was a concentration dependent increase and the maximal response in chromosomal aberrations occurred 24 h after treatment in concentrations used

for both the chemicals.

Tables 6 and 7 and Figures 5 and 6 which are based on these tables, presented the results of micronucleus formation. In all three dose levels the frequency of micronuclei in polychromatic erythrocytes (PCEs) induced by PCP found elevated and consistently above the control levels; a maximum of 1.08 ± 0.003 was recorded at 24 h of C3 to that of normal 0.48 ± 0.50, whereas a maximum of 1.15 ± 0.003 was recorded against 2,4-D. There was a concentration and dose dependent increase in the formation of micronucleated polychromatic erythrocytes (MNPCEs) and the maximum rise in MNPCEs were seen at 24 h of the treatment for either chemical. The P / N Ratio showed no significant change for these chemicals, or the percentage of polychromatic cells. The frequency of PCP induced MNPCEs increased with time and reached at maximum level 24 h after treatment followed by a gradual decrease, however, it was statistically insignificant.

The third parameter chosen was mitotic index(MI) study. It showed a dose and time dependent inhibition in case of PCP and 2,4-D respectively (Figures 7 and 8). A minimum value of 3.01 ± 0.11 was observed for PCP and 3.05 ± 0.12 for 2,4-D. These values in normal was 3.90 ± 0.11 (Table 9). In case of PCP, maximum decrease in mitotic index was seen at 96 h of C3 concentration. A steep decline was recorded as the concentration increased. Both chemicals showed the same trend in mitotic indices as concentration and time increases. Some of the representative chromosomal aberrations and micronucleus formation have been shown in Fig 9 and 10.

Table 4: Chromosomal Aberrations induced by multiple concentrations of Pentachlorophenol (PCP) at different intervals in *Mus musculus*

Duration (h)	Concentration mg/1000g	Aberration Chromatid G	B	MA	Choromosomal G'	B'	T	% Aberration ± SE Chromatid +Gap	Chromatid - Gap	Chromosome + Gap	Chromosome - Gap	% Total Aberration ± SE	% S & P ± SE
12	NC	8	3	4	4	3	3	5.00 ± 0.36	2.33 ± 0.11	3.33±0.20	2.00±0.09	8.33±0.04	2.33±0.11
	SC	3	4	11	5	3	4	6.00 ± 0.03	5.00 ± 0.03	4.00±0.03	2.33±0.02	10.00±0.04	2.66±0.02
	PC	8	15	10	9	6	10	11.00 ± 0.04	8.33 ± 0.03	8.33±0.03	5.33±0.03	17.66±0.05*	9.33±0.03
	C1	9	4	4	5	3	4	5.66 ± 0.03	2.66 ± 0.02	4.00±0.03	2.33±0.02	9.06±0.04	2.33±0.02
	C2	11	4	5	4	2	4	6.33 ± 0.03	2.66 ± 0.02	2.33±0.02	3.66±0.02	10.00±0.04	2.66±0.02
	C3	13	5	7	6	3	5	8.33 ± 0.03	4.00 ± 0.02	2.66±0.02	4.66±0.03	13.00±0.04	2.66±0.02
24	NC	8	3	5	4	2	4	5.33 ± 0.04	2.66 ± 0.14	3.33±0.20	2.00±0.09	8.66±0.04	2.33±0.11
	SC	3	4	10	3	3	4	5.66 ± 0.03	4.66 ± 0.02	3.66±0.02	2.66±0.02	9.00±0.04	2.66±0.02
	PC	4	17	15	10	10	18	12.00± 0.03	10.66 ± 0.04	12.66±0.04	9.33±0.02	24.66±0.04*	10.00±0.03
	C1	12	9	8	9	4	5	9.66 ± 0.04	5.66 ± 0.03	6.33±0.03	3.00±0.02	16.00±0.05*	4.00±0.02
	C2	14	9	12	9	4	4	11.66 ± 0.04	7.00 ± 0.03	5.66±0.03	2.66±0.02	17.33±0.05*	4.33±0.02
	C3	14	10	13	10	4	4	12.33 ± 0.04	7.66 ± 0.03	6.00±0.03	2.66±0.02	18.33±0.05*	4.66±0.03
48	NC	8	3	4	6	3	5	5.00 ± 0.36	2.33 ± 0.11	4.33±0.02	2.33±0.11	9.66±0.04	2.33±0.11
	SC	5	5	7	4	3	7	5.66 ± 0.03	4.00 ± 0.02	3.33±0.02	2.33±0.02	10.33±0.04	2.33±0.11
	PC	6	17	10	13	9	11	11.00 ± 0.04	9.00 ± 0.03	11.00±0.13	6.66±0.03	22.00±0.05*	12.66±0.04
	C1	11	4	4	8	2	4	6.33 ± 0.03	2.66 ± 0.02	4.66±0.02	2.00±0.03	11.00±0.04	2.33±0.02
	C2	6	4	6	10	2	3	5.33 ± 0.03	3.33 ± 0.02	5.00±0.03	1.66±0.02	10.33±0.04	4.11±0.02
	C3	10	4	3	4	3	17	11.66 ± 0.03	7.00 ± 0.03	5.66±0.03	2.66±0.03	17.33±0.05*	4.00±0.02
96	NC	7	3	5	4	3	3	4.66 ± 0.36	2.33 ± 0.11	3.33±0.20	2.00±0.29	8.33±0.04	2.00±0.09
	SC	2	5	8	2	3	6	5.00 ± 0.03	4.33 ± 0.02	3.66±0.02	3.00±0.02	8.66±0.04	2.33±0.02
	PC	9	12	18	8	9	12	13.00 ± 0.04	10.00 ± 0.03	9.66±0.03	7.00±0.03	22.66±0.05*	10.33±0.28
	C1	12	3	3	7	3	5	6.00 ± 0.03	2.00 ± 0.02	5.00±0.03	2.66±0.02	11.00±0.04	2.33±0.02
	C2	2	5	13	2	5	6	6.66 ± 0.03	6.00 ± 0.03	4.00±0.03	3.33±0.02	11.00±0.04	4.00±0.02
	C3	9	4	5	5	4	4	6.00 ± 0.03	3.00 ± 0.02	4.33±0.03	2.66±0.02	10.33±0.04	2.33±0.02

NC, Normal Control, Tap Water; SC, Solvent Control, Ethanol; PC, Positive Control. Cyclophosphamide 40mg / 1000g; C1, 7mg / 1000g; C2, 15mg / 1000g;C3, 20mg / 1000g; G, Gap. B, Break, MA, T S&P, multiple aberration, translocation and stickiness & pulvelization;* Significant

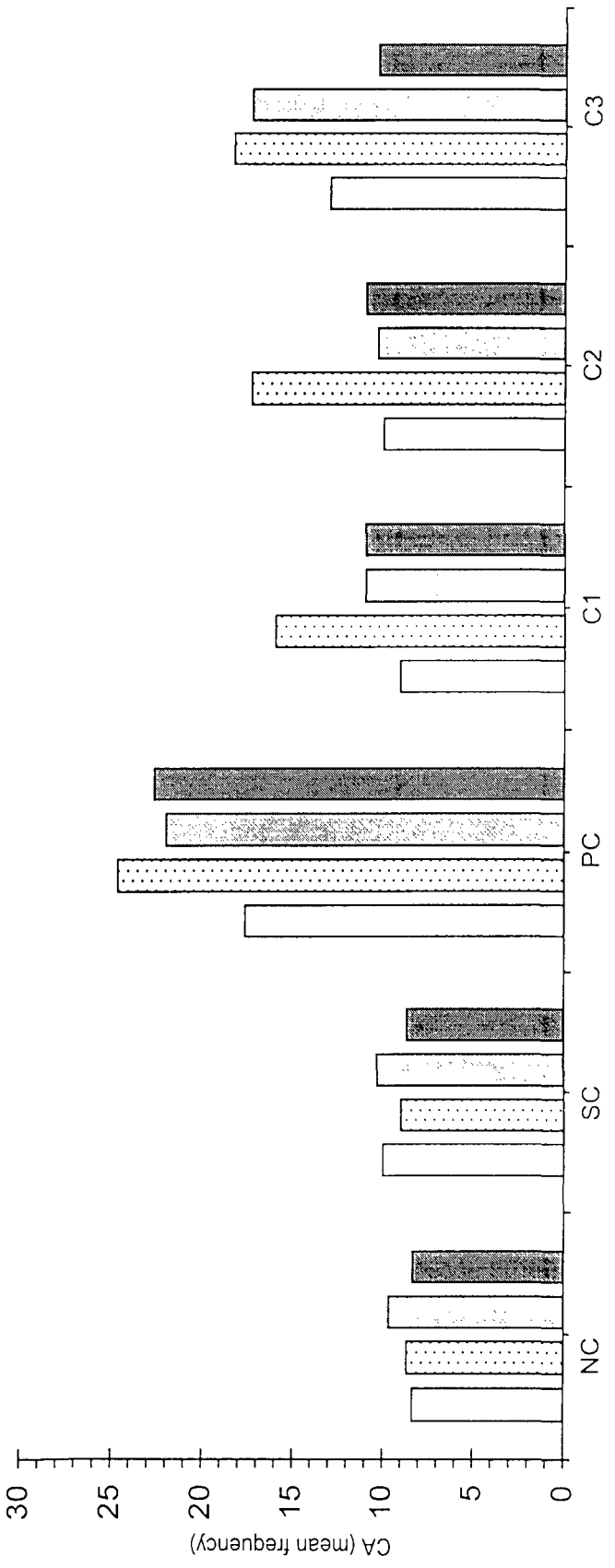


Figure. 1: **Multiple concentration and duration related profiles of Chromosomal Aberration (CA) by Pentachlorophenol (PCP) in *Mus musculus***

NC, Normal Control; Tap Water. SC, Solvent Control, Ethanol; Positive Control, Cyclophosphamide 40mg/1000g; C1 - 7mg / 1000g; C2 - 15mg / 1000; C3 - 20mg / 1000g

□12 □24 □48 ▨96h

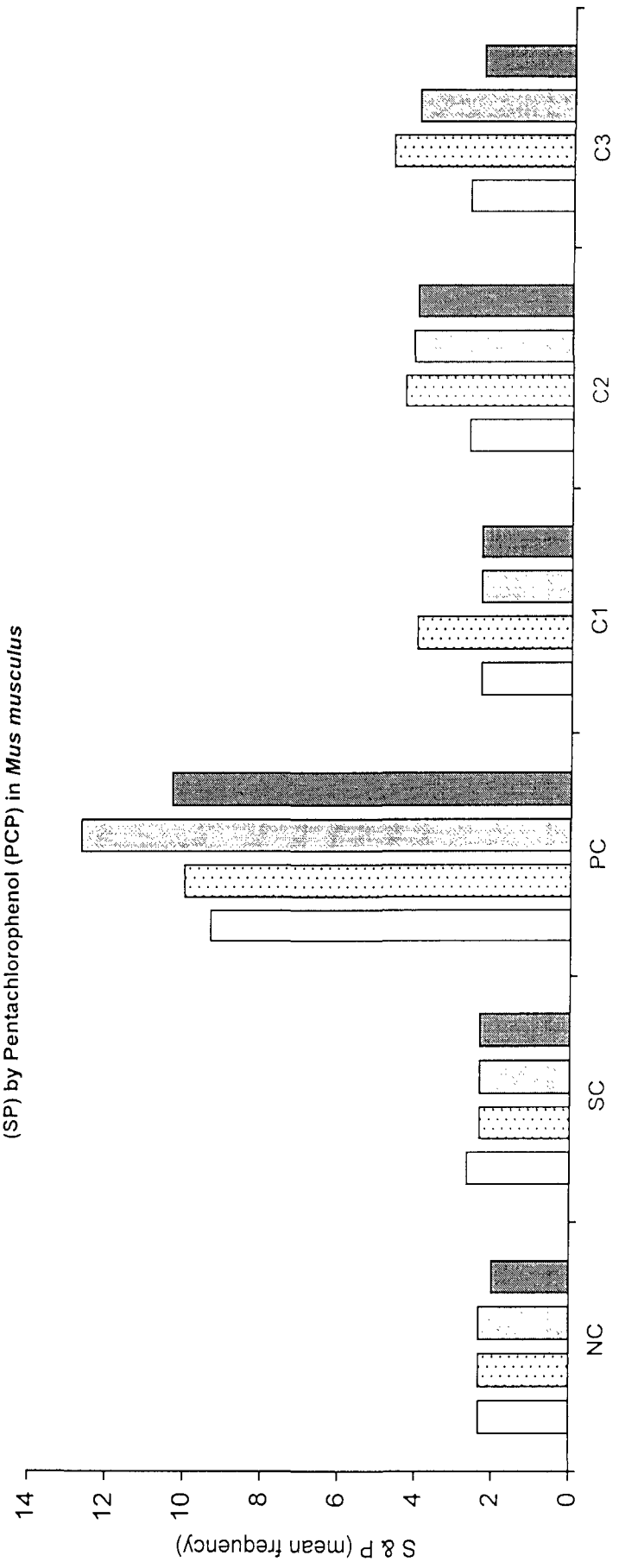


Fig. 2: Multiple Concentration and duration depedent profiles of Stickiness and pulverisation (SP) by Pentachlorophenol (PCP) in ***Mus musculus***

NC, Normal Control, Tap Water; SC, Solvent Control, Ethanol; Positive Control, Cyclophosphamide 40mg/1000g; C1 - 7mg / 1000g; C2 - 15mg / 1000; C3 - 20mg / 1000g

Table 5: Chromosomal Aberration Induced by multiple concentration of 2,4-Dichlorophenoxyacetic acid (2,4-D) at different intervals in *Mus musculus*

Duration (h)	Concentration mg/1000g	Aberration: Chromatid G	B	MA	Choromosomal G'	B'	T	% Aberration ± SE: Chromatid +Gap	Chromatid -Gap	Chromosome +Gap	Chromosome -Gap	% Total Aberration ±SE	% S & P ± SE
12	NC	8	3	4	4	2	4	5.00 ± 0.36	2.33 ± 0.11	3.33±0.20	2.00±0.09	8.33±0.04	2.33±0.11
	SC	4	4	8	4	2	3	5.33 ± 0.03	4.00 ± 0.02	3.66±0.02	1.66±0.02	8.33±0.04	4.00±0.02
	PC	8	5	0	9	6	10	11.00± 0.04	8.33 ± 0.03	8.33±0.03	5.33±0.03	16.00±0.05*	9.33±0.03
	C1	11	3	5	4	2	4	6.33 ± 0.04	2.33 ± 0.02	3.00±0.02	2.00±0.02	9.66±0.04	2.66±0.02
	C2	22	3	4	3	3	3	3.00 ± 0.03	6.33 ± 0.02	2.66±0.03	4.33±0.02	10.00±0.04	3.00±0.02
	C3	10	4	5	5	4	4	9.66 ± 0.04	2.33 ± 0.02	3.00±0.02	2.00±0.02	13.66±0.04	3.33±0.02
24	NC	8	3	5	4	2	4	5.33 ± 0.40	2.66 ± 0.14	3.33±0.20	2.00±0.09	8.66±0.04	3.33±0.11
	SC	6	4	8	4	3	4	6.00 ± 0.03	4.00 ± 0.02	3.66±0.02	2.33±0.02	8.66±0.04	3.00±0.02
	PC	4	5	17	10	14	14	12.00± 0.03	10.66± 0.04	12.66±0.04	9.33±0.02	21.33±0.05*	13.33±0.03
	C1	13	3	6	4	2	5	7.03 ± 0.03	3.00 ± 0.02	3.33±0.02	2.33±0.02	11.00±0.04	3.33±0.02
	C2	11	8	16	5	3	4	8.00 ± 0.03	4.66 ± 0.03	6.00±0.03	3.33±0.02	14.00±0.05*	3.66±0.02
	C3	10	8	6	8	4	6	11.60 ± 0.04	8.00 ± 0.03	4.00±0.03	2.33±0.02	15.66±0.05*	4.00±0.02
48	NC	8	4	3	6	2	5	5.00 ± 0.36	2.33 ± 0.11	2.33±0.20	4.33±0.29	9.33±0.04	3.00±0.11
	SC	3	6	6	4	3	5	5.00 ± 0.03	4.00 ± 0.02	4.00±0.03	2.66±0.02	9.33±0.04	3.00±0.28
	PC	6	16	11	13	9	11	11.00 ± 0.04	9.00 ± 0.03	1.00±0.13	6.66±0.03	22.00±0.05*	12.60±0.04
	C1	12	4	5	3	3	8	7.00 ± 0.03	3.02 ± 0.02	5.00±0.03	3.66±0.27	11.66±0.04	2.66±0.02
	C2	3	5	14	2	4	10	7.33 ± 0.03	6.33 ± 0.03	4.66±0.03	3.66±0.02	12.66±0.04	2.66±0.03
	C3	15	6	9	9	5	2	10.00 ± 0.04	5.00 ± 0.03	5.33±0.03	2.33±0.02	15.33±0.05*	2.00±0.02
96	NC	7	4	2	4	2	4	4.66 ± 0.36	2.33 ± 0.11	3.33±0.20	2.00±0.29	7.66±0.04	2.66±0.09
	SC	9	5	4	6	3	5	6.33 ± 0.03	3.33 ± 0.02	4.66±0.33	2.66±0.02	10.66±0.04	2.66±0.28
	PC	9	16	4	9	9	12	13.00 ± 0.04	10.00 ± 0.03	9.66±0.03	7.00±0.03	19.66±0.05*	10.00±0.04
	C1	18	5	5	3	3	4	6.00 ± 0.03	3.33 ± 0.02	3.33±0.02	2.33±0.02	12.66±0.04	2.33±0.02
	C2	13	8	12	4	3	5	5.66 ± 0.03	3.00 ± 0.02	4.00±0.03	3.66±0.26	9.66±0.04	2.66±0.04
	C3	8	6	3	1	2	9	11.04 ± 0.03	6.66 ± 0.03	4.00±0.03	2.66±0.02	15.00±0.05*	2.00±0.02

NC, Normal Control, Tap Water; SC, Solvent Control, Distilled Water; PC, Positive Control, Cyclophosphamide 40mg / 1000g; C1, 25mg / 1000g; C2,50mg / 1000g; C3, 75mg / 1000g; G, G', Gap; B, B', break MA, T S&P, multiple aberration, translocation and stickness & pulvalization;* Significant

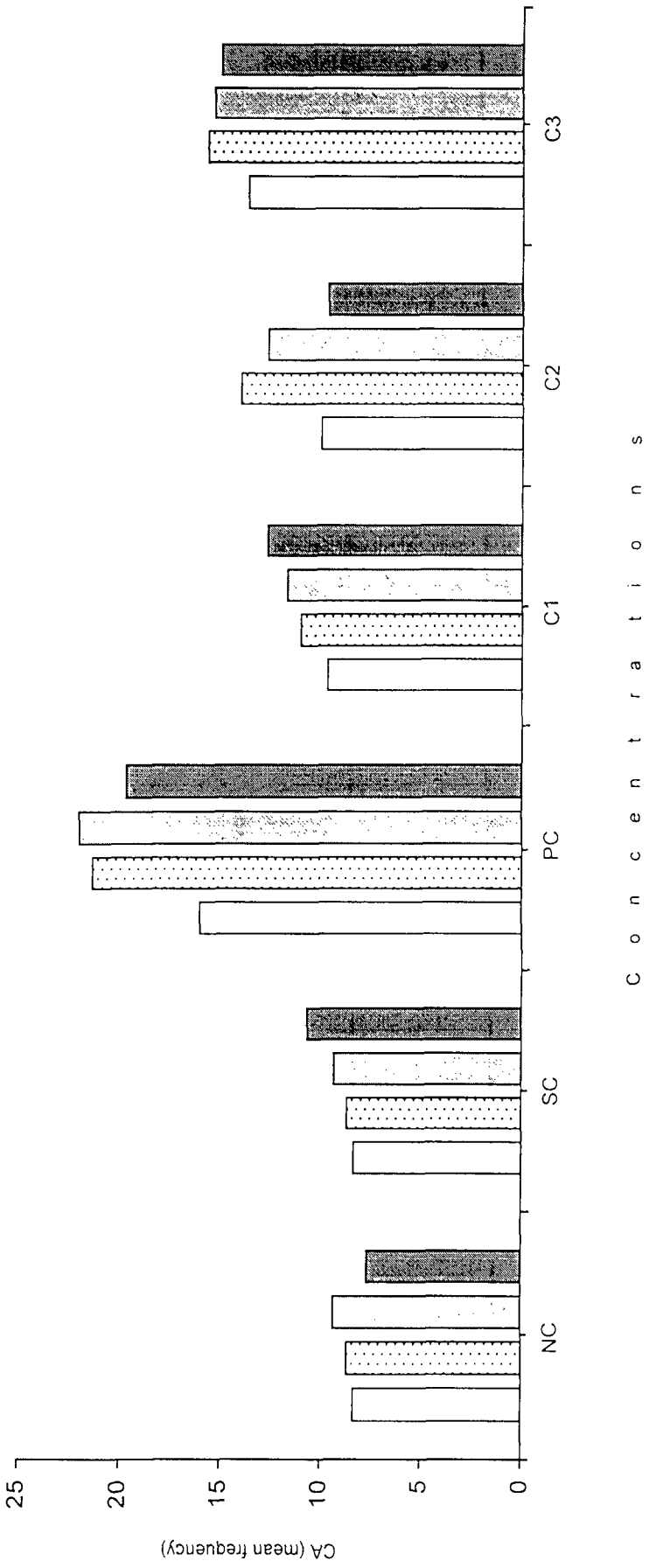
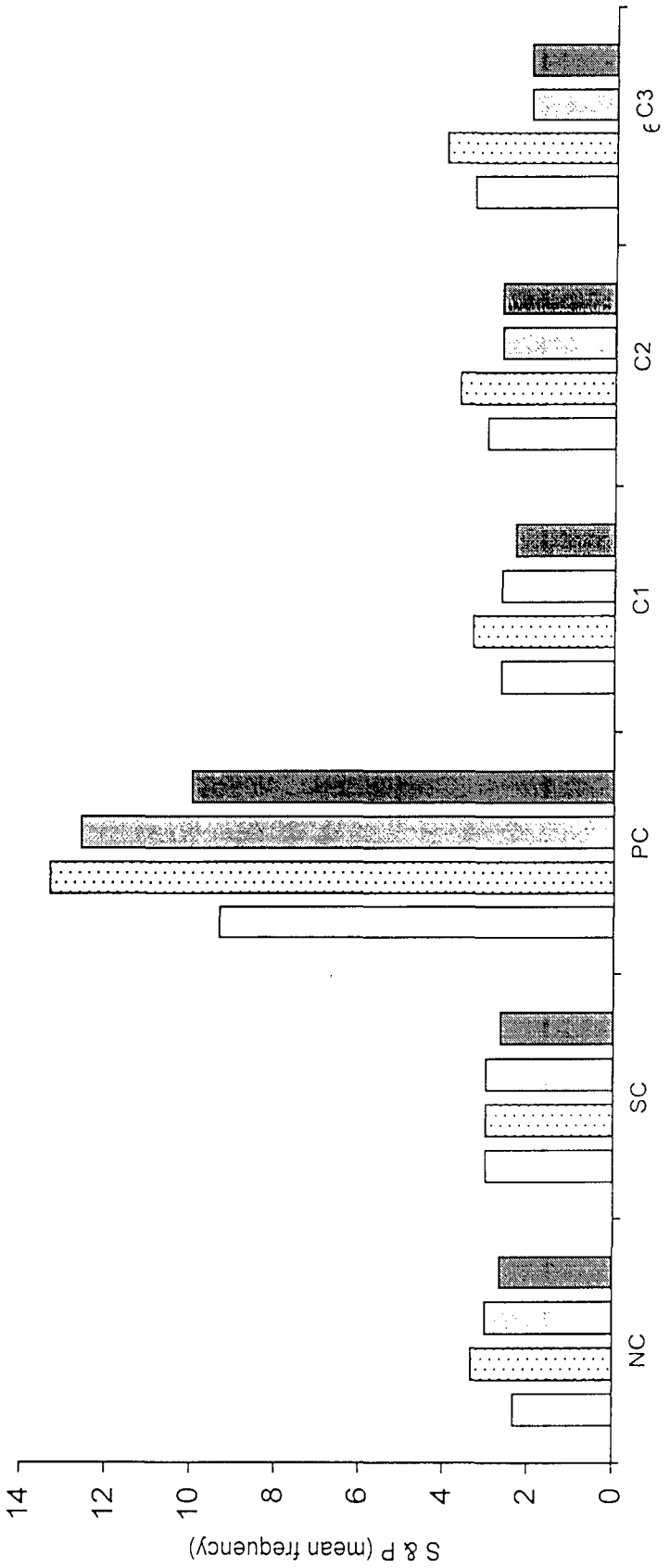


Figure. 3: **Multiple concentration and duration dependent profiles of Chromosomal Aberration (CA) by 2, 4 Dichlorophenoxyacetic acid (2, 4-D) in *Mus musculus***

Figure. 4: Muliple Concentration and duration dependent profiles of Stickness and pulverisation (SP) by 2, 4 Dichlorophenoxyacetic Acid (2, 4-D) in *Mus musculus*



NC, Normal Control, Tap Water; SC, Solvent Control, Distilled Water; Positive Control, Cyclophosphamide 40 mg/1000g; C1 - 25mg / 1000g; C2 - 50mg / 1000; C3 - 75mg / 1000g.

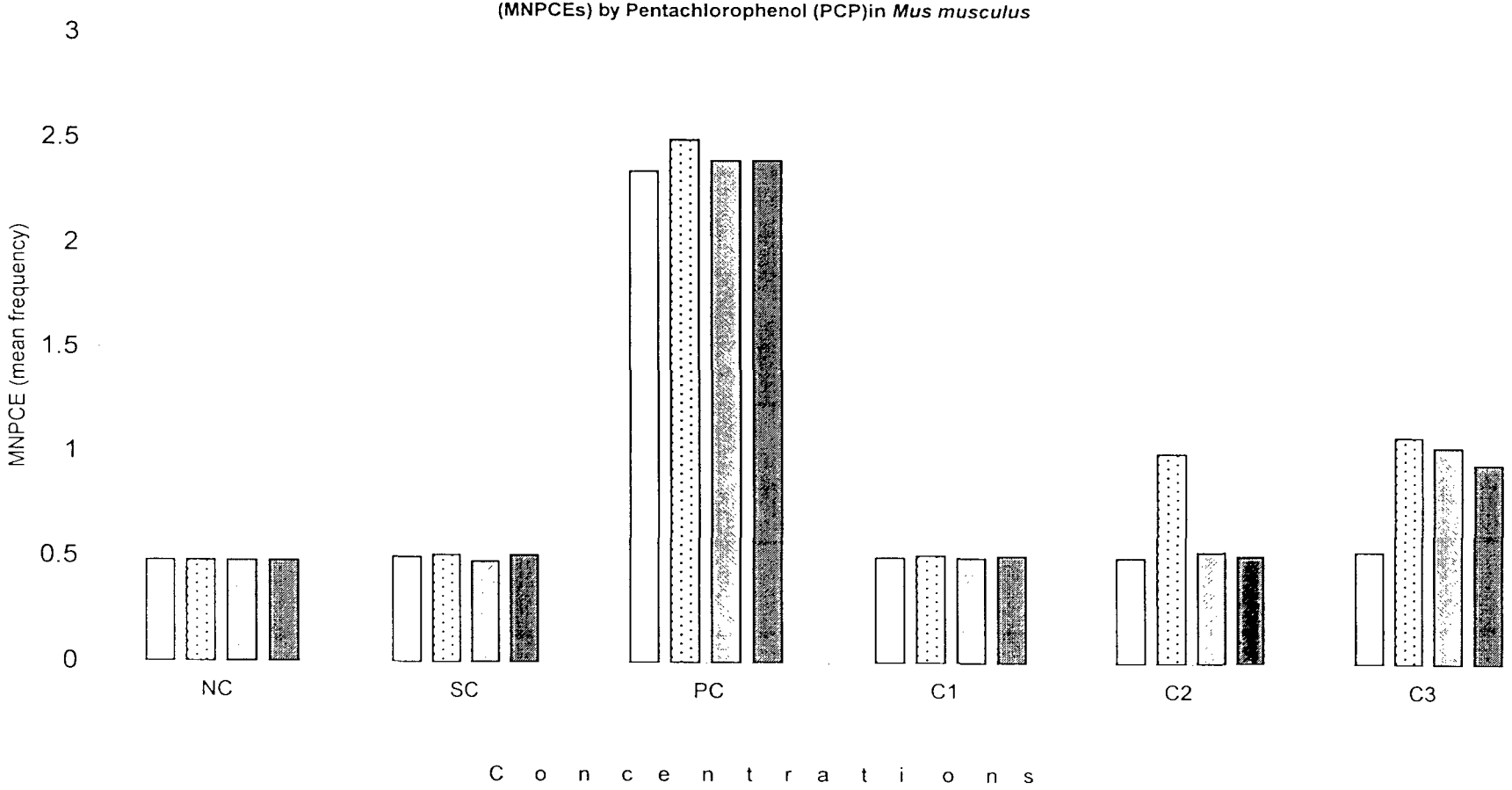
□12 □24 □48 ■96h

Table 6 : Incidence of micronucleus formation in Polychromatic erythrocytes (PCE) at multiple concentrations of Pentachlorophenol (PCP) at different time intervals in *Mus musculus*

Duration h	Concentration mg/1000g	% PCE	% NCE	% MN in PCE± SEM	% MN in NCE ± SEM	P/N ratio
12	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.010	1.04
	SC	50.45	49.57	0.50 ± 0.002	0.16 ± 0.001	1.01
	PC	52.48	47.51	2.35 ± 0.004*	0.68 ± 0.002	1.00
	C1	50.84	49.15	0.50 ± 0.002	0.17 ± 0.001	1.03
	C2	50.89	49.1	0.50 ± 0.002	0.19 ± 0.001	1.03
	C3	51.93	48.18	0.53 ± 0.002	0.21 ± 0.001	1.08
24	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.010	1.04
	SC	50.45	49.54	0.51 ± 0.002	0.15 ± 0.001	1.01
	PC	50.07	49.92	2.50 ± 0.004*	0.66 ± 0.002	1.01
	C1	50.85	49.14	0.51 ± 0.002	0.18 ± 0.001	1.03
	C2	50.76	49.23	1.00 ± 0.003*	0.17 ± 0.001	1.03
	C3	50.46	49.53	1.08 ± 0.003*	0.23 ± 0.001	1.01
48	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.100	1.04
	SC	50.43	49.56	0.48 ± 0.002	0.18 ± 0.001	1.01
	PC	52.96	47.03	2.40± 0.004*	0.67 ± 0.002	1.12
	C1	51.63	48.74	0.50 ± 0.002	0.17 ± 0.001	1.06
	C2	51.24	48.75	0.53 ± 0.002	0.21 ± 0.001	1.05
	C3	51.01	46.59	1.03 ± 0.003*	0.20 ± 0.001	1.04
96	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.010	1.04
	SC	50.43	48.71	0.51 ± 0.002	0.18 ± 0.001	1.03
	PC	51.01	48.99	2.40 ± 0.004*	0.66 ± 0.002	1.04
	C1	52.03	47.96	0.51 ± 0.002	0.16 ± 0.001	1.08
	C2	51.58	48.41	0.50 ± 0.002	0.17 ± 0.001	1.06
	C3	53.00	46.55	0.95 ± 0.003*	0.20 ± 0.001	1.12

NC, Normal Control, Tap Water; SC, Solvent Control, Ethanol; PC, Positive Control, Cyclophosphamide 40mg / 1000g; C1, 7mg / 1000g; C2, 15mg / 1000g; C3, 20mg / 1000g; PCE, Polychromatic Erythrocytes; NCE, Normochromatic Erythrocytes: SEM, Standard Error Mean

Figure. 5: Dose-duration dependent profiles of Micronucleated Polychromatic Erythrocytes (MNPCEs) by Pentachlorophenol (PCP)in *Mus musculus*



NC, Normal Control, Tap Water; SC, Solvent Control, Ethanol; Positive Control, Cyclophosphamide 40mg/1000g; C1 - 7mg / 1000g; C2 - 15mg / 1000; C3 - 20mg / 1000g

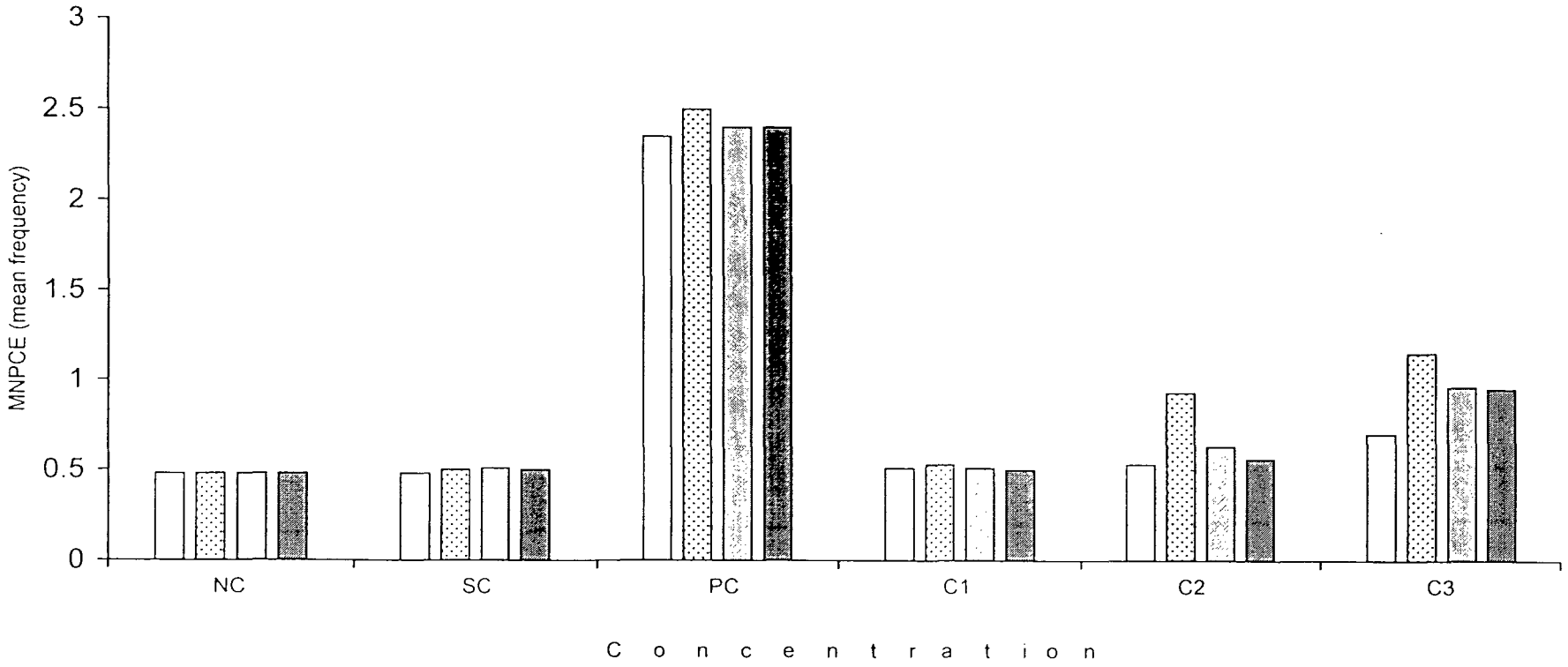
□12 □24 □48 ▨96h

Table 7: Micronucleus formation in Polychromatic erythrocytes (PCE) at multiple concentrations of 2, 4-Dichlorophenoxyacetic Acid (2, 4-D) at different time intervals in *Mus musculus*

Duration h	Concentration mg/1000g	% PCE	% NCE	% MN in PCE± SEM	% MN in NCE ± SEM	P/N ratio
12	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.010	1.04
	SC	51.43	46.56	0.48 ± 0.002	0.18 ± 0.001	1.01
	PC	52.48	47.51	2.35 ± 0.004*	0.68 ± 0.002	1.10
	C1	51.29	48.70	0.51 ± 0.002	0.19 ± 0.001	1.05
	C2	52.26	47.73	0.53 ± 0.002	0.21 ± 0.001	1.09
	C3	50.43	49.53	0.70 ± 0.002	0.27 ± 0.001	1.01
24	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.010	1.04
	SC	50.45	49.57	0.50 ± 0.002	0.16 ± 0.001	1.01
	PC	50.07	49.92	2.50 ± 0.004*	0.66 ± 0.002	1.01
	C1	50.97	49.02	0.53 ± 0.002	0.21 ± 0.001	1.03
	C2	50.05	49.94	0.93 ± 0.003*	0.20 ± 0.001	1.00
	C3	50.11	49.88	1.15 ± 0.003*	0.25 ± 0.001	1.00
48	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.010	1.04
	SC	50.45	49.54	0.51 ± 0.002	0.15 ± 0.001	1.01
	PC	52.96	47.03	2.40± 0.004*	0.67 ± 0.002	1.12
	C1	50.17	47.82	0.51 ± 0.002	0.21 ± 0.001	1.09
	C2	50.16	49.83	0.63 ± 0.002	0.23 ± 0.001	1.00
	C3	50.66	48.33	0.96 ± 0.003*	0.27 ± 0.001	1.02
96	NC	51.01	48.98	0.48 ± 0.500	0.17 ± 0.010	1.04
	SC	50.45	49.54	0.50 ± 0.002	0.16 ± 0.001	1.01
	PC	51.01	48.99	2.40 ± 0.004*	0.66 ± 0.001	1.04
	C1	52.07	46.18	0.50 ± 0.002	0.19 ± 0.001	1.15
	C2	51.18	48.81	0.56 ± 0.002	0.22 ± 0.001	1.04
	C3	52.81	47.18	0.95 ± 0.003*	0.24 ± 0.001	1.11

NC, Normal Control, Tap Water; SC, Solvent Control, Ethanol; PC, Positive Control, Cyclophosphamide 40mg / 1000g; C1, 25mg / 1000g; C2, 50mg / 1000g; C3, 75mg / 1000g, PCE, Polychromatic Erythrocytes; NCE, Normochromatic Erythrocytes. SEM Standard Error Mean: *Significant.

Figure. 6 : Dose-duration dependent profiles of Micronucleated Polychromatic Erythrocytes (MNPCEs) by 2, 4 Dichlorophenoxyacetic acid (2, 4-D) in *Mus musculus*



NC, Normal Control, Tap Water; SC, Solvent Control, Distilled Water; Positive Control, Cyclophosphamide 40mg/1000g; C1 - 25mg / 1000g; C2 - 50mg / 1000; C3 - 75mg / 1000g

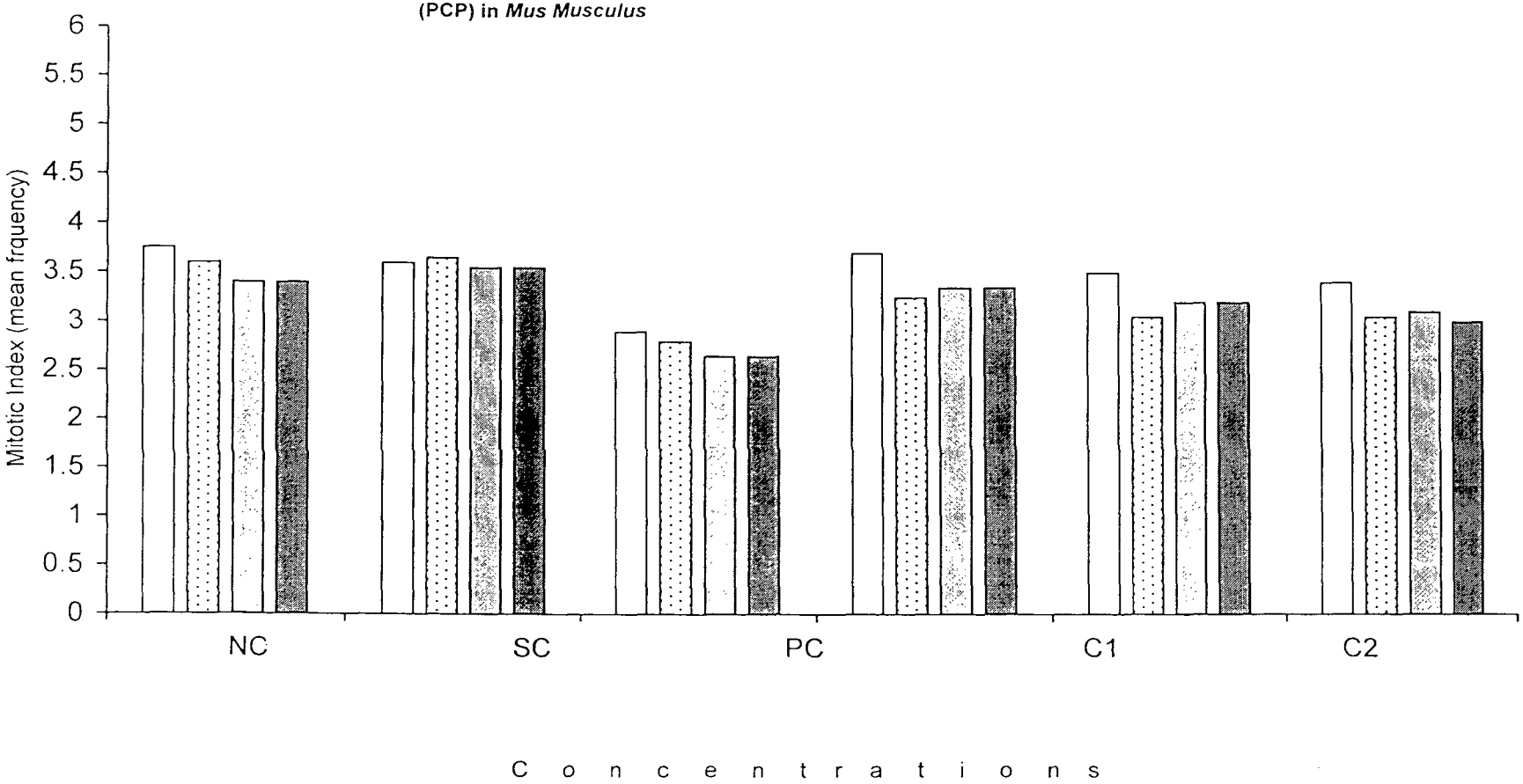
□12 ▨24 □48 ▩96h

Table 8: Mitotic Indices in response to various concentrations of Pentachlorophenol (PCP) at different time intervals in *Mus musculus*

Concentration mg/1000g	Mitotic Index ± SE 12h	24h	48h	96h
NC	3.75±0.11	3.60±0.11	3.60±0.11	3.50±0.11
SC	3.60±0.11	3.65±0.11	3.55±0.11	3.55±0.11
PC	2.90±0.11	2.80±0.11	2.65±0.11	2.65±0.11
C1	3.70±0.11	3.45±0.11	3.35±0.11	3.25±0.11
C2	3.50±0.11	3.20±0.11	3.05±0.11	3.03±0.11
C3	3.40±0.11	3.10±0.11	3.05±0.11	3.01±0.11

NC, Normal Control, Tap Water; SC, Solvent Control, Distilled Water; Positive Control, Cyclophosphamide 40 mg/1000g; C1 - 7mg / 1000g; C2 - 15mg / 1000; C3 - 20mg / 1000g

Figure 7: Multiple Concentrations and duration dependent profiles of mitotic index (MI) by Pentachlorophenol (PCP) in *Mus Musculus*



NC, Normal Control, Tap Water; SC, Solvent Control, Ethanol; Positive Control, Cyclophosphamide 40 mg/1000g; C1 - 7mg / 1000g; C2 - 15mg / 1000; C3 - 20mg / 1000g

□12 □24 □48 ▩96h

Table 9: Mitotic indices in response to various concentrations of 2.4-D at different time intervals in *Mus musculus*

Concentration mg/1000g	Mitotic Index ± SE 12h	24h	48h	96h
NC	3.71±0.11	3.90±0.11	3.60±0.11	3.60±0.11
SC	3.60±0.11	3.65±0.11	3.55±0.11	3.55±0.11
PC	2.90±0.11	2.80±0.11	2.65±0.11	2.65±0.11
C1	3.70±0.11	3.70±0.11	3.60±0.11	3.50±0.11
C2	3.40±0.11	3.45±0.11	3.25±0.11	3.10±0.11
C3	3.20±0.11	3.05±0.12	3.05±0.13	3.10±0.14

NC, Normal Control, Tap Water; SC, Solvent Control, Ethanol; Positive Control, Cyclophosphamide g/1000g; C1 - 25mg / 1000g; C2 - 50mg / 1000; C3 - 75mg / 1000g

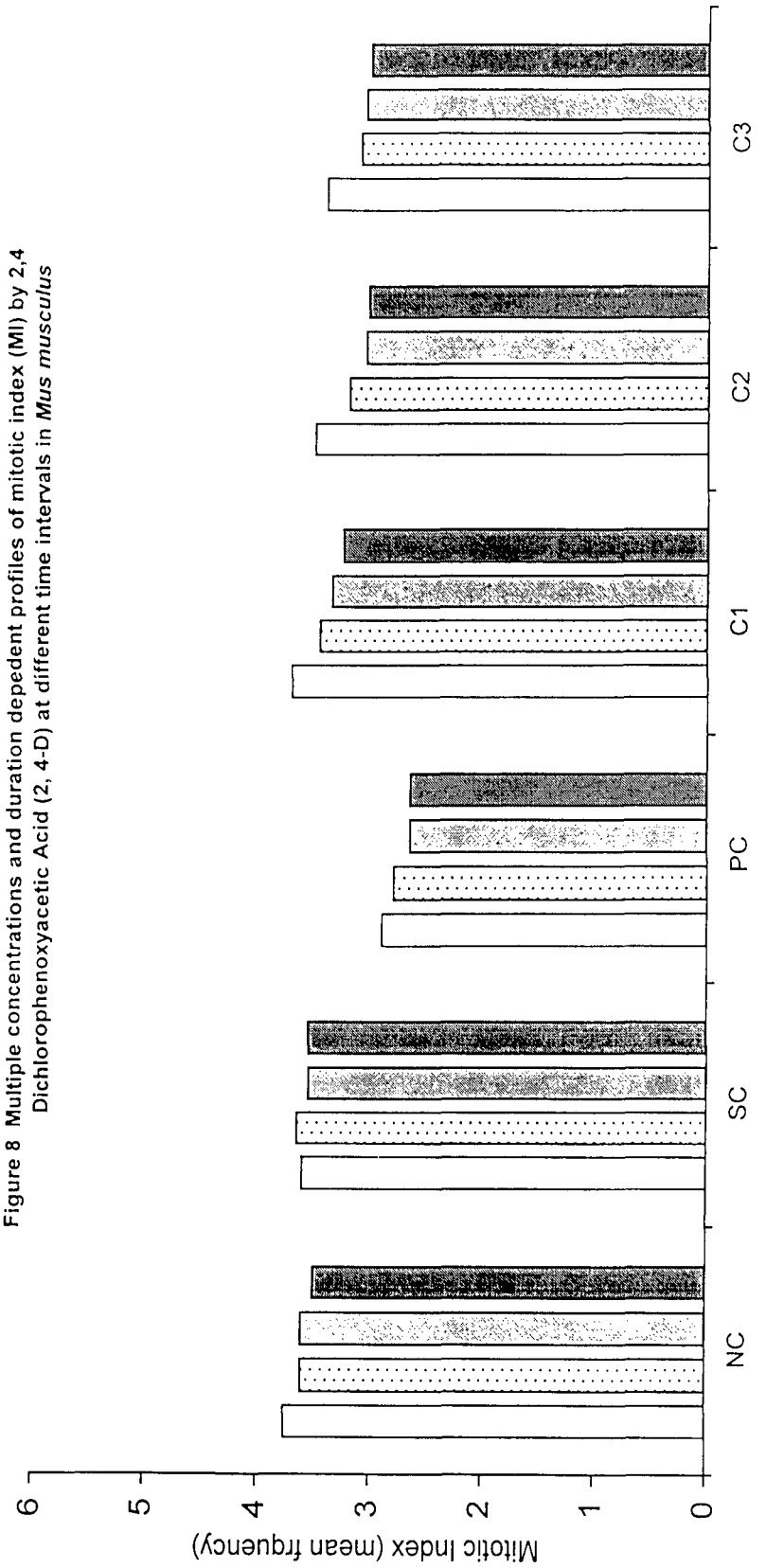


Figure 8 Multiple concentrations and duration depedent profiles of mitotic index (MI) by 2,4 Dichlorophenoxyacetic Acid (2, 4-D) at different time intervals in *Mus musculus*

NC, Normal Control, Tap Water; SC, Solvent Control, Distilled Water; Positive Control, Cyclophosphamide mg/1000g; C1 - 25mg / 1000g; C2 - 50mg / 1000; C3 - 75mg / 1000g
□12 □24 □48 ▣96h

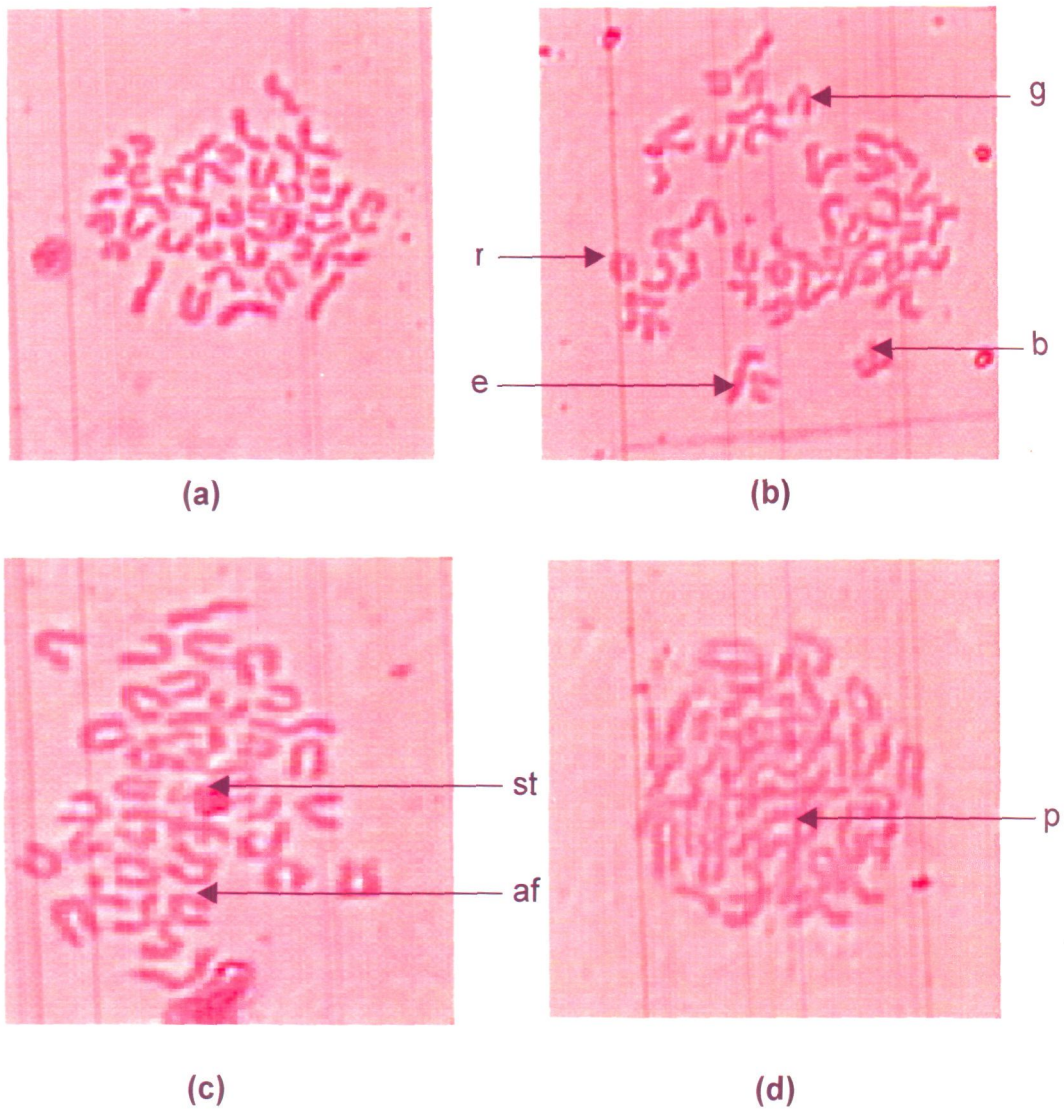


Figure 9: Representative Photomicrograph of normal karyotype (**a**) and chromosomal aberrations (**b,c** and **d**)observed in bone marrow cells of *Mus musculus* by PCP and 2,4-D.

g- gap, b- break, af acentric fragment, e- exchange, st- stickiness, p- pulverization r- ring chromosome

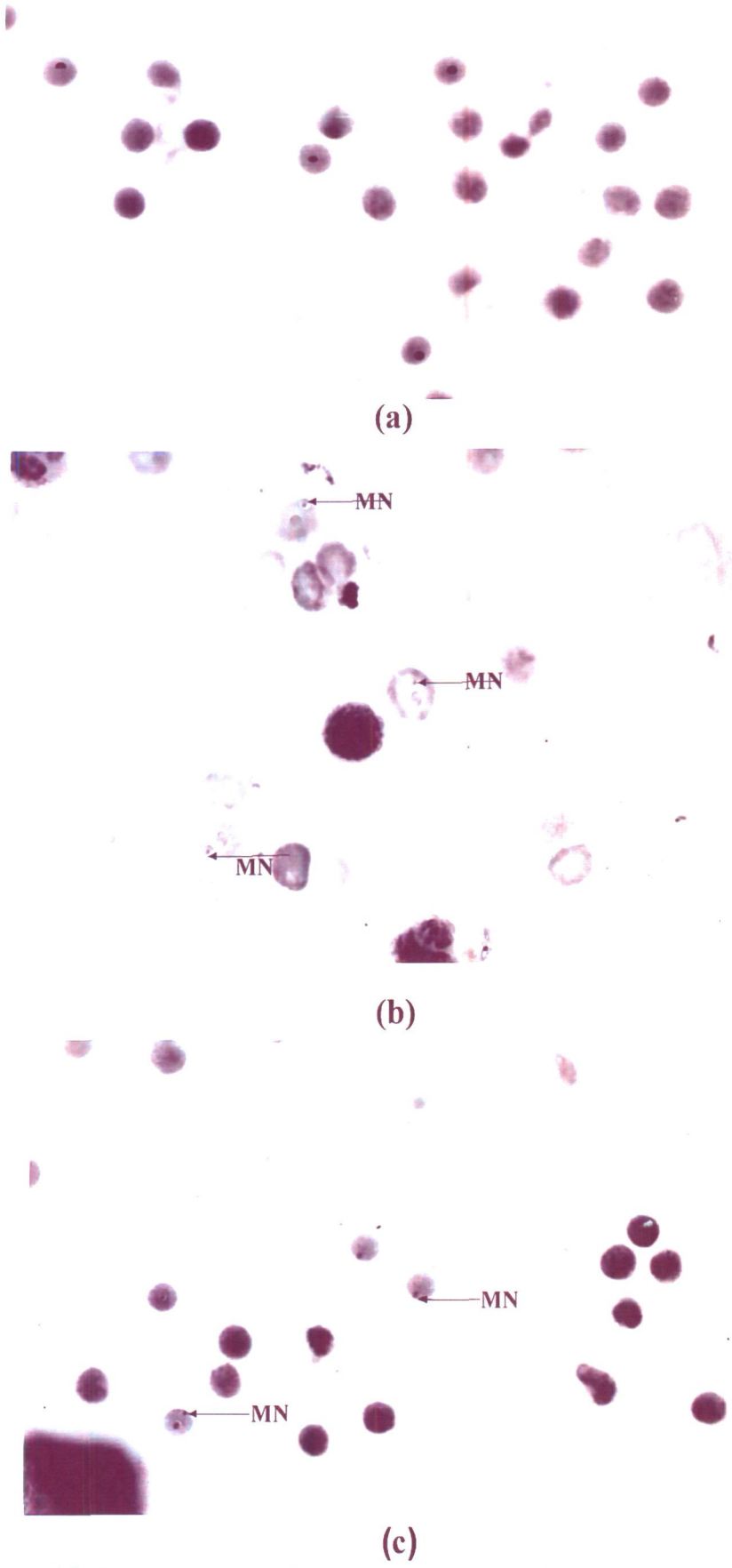


Figure10: Representative Photomicrographs of micronucleus formation in polychromatic erythrocytes in bone marrow cells of *Mus musculus* by PCP and 2,4-D.

(a) Normal

(b) MNPCEs : Micronucleated polychromatic erythrocytes

(c) MNNCEs : Micronucleated normochromatic erythrocytes

DISCUSSION.

Genotoxicity tests are vital on a routine basis for most categories of chemicals such as pesticides, drugs, food additives etc (Kramers et al., 1991). Out of a battery of parameters, short term tests in mammals have received much attention as a protective pre requisite to human life. The present study was conducted by three such tests; the in vivo bone marrow chromosomal assay, micronucleus assay and mitotic index study to evaluate mutagenic potential of chemicals namely PCP and 2,4-D.The induction of chromosome and chromatid type deletions, exchanges and MN in the bone marrow cells of mice observed in our experiments confirmed the clastogenic potential as well indication of as spindle poisoning action of PCP and 2,4-D. However, the P/N ratio suggested a lack of cytotoxic effect of the chemicals at the present concentrations.

There are two important factors of micronucleus formation; due to chromosome breakage and spindle inhibition (Schmid, 1976). The findings that chemicals with clastogenic activities induce micronuclei has generally been accepted as an indication of micronuclei, that are derived from chromatid or chromosome fragments or lagging chromosomes. Nevertheless, only few studies could focuss on the mechanism of micronucleus formation. Evans et al., (1959), showed that micronuclei are resulted from chromosomal aberrations induced by fast neutrons and gamma rays in the root tips of *Vicia faba*. In an in vitro study, Countryman and Heedle (1976) showed that dosimetric aspects of micronucleated cells agree with those expected from metaphase analysis of aberrations in cultured human cells. The relationship between chromosomal aberrations and micronuclei could be demonstrated only on the basis of theoretical considerations (Heedle and Carrano, 1977).

Chromatid type breaks were seen more frequently than chromosome type of breaks in the metaphase plate analysis of both the chemicals. This indicated that damage affected the DNA strands in the late S phase or after the DNA has replicated or duplicated (Bird et al., 1982). Similarly Pilocarpine-induced significant c-mitosis and chromosomal aberrations like breaks, exchanges,

stickiness and pulverization in dose dependent manner endorsed this trend. The types of breaks brought about by the present test chemicals indicate that they are active in G1 and S phase of cell cycle. Other drugs also found to inhibit DNA synthesis in peripheral blood mononuclear cells (Artz et al., 1989). The time response study, as observed in the present experiments indicate that frequency of stickiness and pulverisation increased from 12 to 24h followed by rapid decrease at 48h of the treatment. This fully agrees with the statement of Savage that stickiness is a transient phenomenon.

Various biochemical and related interpretations are given by many investigators to explain the induction of stickiness (Darlington, 1942; DeRobertis et al., 1974; Giri et al., 1981). From these different views, it is inferred that chemically induced stickiness is very complicated phenomenon involving properties of chromosomes. Chromatid breaks were seen more frequently than chromosome breaks in metaphase plate analysis, indicating damage affecting the DNA strand in its late S phase.

That the significant increase in aberration at 24h of the treatment of the chemical and the gradual decrease later on, indicate that these chemicals were active during this period. The decline in the frequencies of aberrations observed in the later time intervals could be due to various reasons like elimination of chemical or its metabolite from the body, repair of damaged genetic material, elimination of cell's chromosome with damaged genetic material, and the inactivation of the chemical or its metabolites (Shyama et al., 1991). The prevalence of endomitotic reduplication in PCP exposed bone marrow was a noteworthy observation. Endomitotic reduplication is a specialized process where doubling of genetic material have the tendency of the cells to pass from G2 to the next S phase without undergoing cell division. Microtubular spindle disturbances are considered important to this process with implication of possible aneuploidy (Dean and Danford, 1984). A variety of chemicals are known to induce endomitotic reduplication that influence replication (Sutou and Arai, 1975; Speit et al., 1987; Sognier and Hittleman, 1986; Caligo and Rainaldi, 1987; Bean et al., 1992; Gurr et al., 1933).

Anovolar 21, an oral contrareceptive which contains progestrin norethisterone acetate, induces considerable stickiness in chromosomes of plants and animals (Hakeem and Amer 1965; Kabarity and Mazrooei, 1984; Shyama et al 1991). The same was corborated by the dose of Tris-azaridinyl-phosphine-oxide (TEPA) (Adler et al., 1971) at 10mg/Kg and for MC at 0.35mg/Kg (Manyak and Schlerermacher, 1973). On the other hand, the data with trimethyiphosphate (TMP) showed a plateau between 24 and 48h (Adler et al., 1971). Similarly cytocine arabinoside showed aberration maximized in bone marrow of mice at 6h after treatment (Wobus et al., 1978). The time of maximal aberration may vary from chemical to chemical; most chemical mutagens act specifically during a particular part of the cell cycle (Bender et al., 1974).

Fluchloralin, a slightly hazardous compound induced chromosome fragments, bridges, micronuclei and chromosome vagrancy (Rao et al., 1990). CA and MN assays indicated that fluchloralin at higher concentrations positively damage human genome. With high doses, the two injections recommended by standard protocols are not likely to maximize the response of micronuclei test. No treatment given less than 10h before bone marrow sampling gave a clear-cut response in micronucleus assay. Therefore, using a single treatment and different intervals best fullfills the requirements for a sensitive micronucleus test in the battery of short-term tests applied to detect chemical mutagens.

The P/N ratio is an indicator of inhibition of nucleated erythropoietic cell divisions, and is recommended in some guidelines for micronucleus test (EPA, 1952; EEC, 1979; OECD, 1983; Gene Tox Program). Such changes in the P/N ratio has been suggested due to unbalanced changes in number of both PCE and NCE (Suzuki et al., 1989,1993). The P/N ratio may be altered in several ways- acceleration of differentiation of erythrocytes from erythroblasts, inhibition of erythroblast division, recovery of erythroblast division are some of them. Lead nitrate treatment for example, resulted in a spurt in the erythropoiesis as was evidenced by a significant increase in the ratio of Polychromatic to Normochromatic erythrocytes ratio (Jagetia et al., 1998). The frequeny of micronuclei in this case did not show a dose related increase in mice. However,

the increase in micronuclei may be owing to the induction of DNA strand breaks by lead nitrate. Lead nitrate treatment induced DNA strand breaks significantly in fresh water mussel foot (Black et al., 1996).

Genotoxicity studies also provide a means of indirectly detecting the presence of metabolically formed reactive intermediates. The result from a wide variety of in vitro and in vivo genotoxicity bioassays indicate that 2,4-D has little, if any, genotoxicity potential. These findings are consistent with the lack of evidence for the presence of reactive intermediates obtained in metabolism studies and also consistent with the lack of structural features of 2,4-D which are known to be associated with the biological or chemical reactivity.

The results of the most of the bacterial mutagenicity tests including *Salmonella typhimurium* and *Escherichia coli* have been eventually negative (Zetterberg, 1978). Tests conducted with and without the presence of rat S9 (post mitchondrial liver homogenate) further gave negative results (Zetterberg et al., 1977; Simmon et al., 1977; Waters et al., 1980). In mammalian cell lines in which unscheduled DNA repair and sister chromatid exchange (SCE) bioassays were studied, mixed results are reported (Styles, 1977; Waters et al., 1980; Calusen et al., 1990; Jacobi and Witte, 1991). The marginally positive results often occurred only at toxic doses (Korte and Jalal, 1982; Clausen et al., 1990).

The in vitro mutagenicity / genotoxicity data suggest that 2,4-D has no or very little genotoxic potential. This conclusion was reached in a review of the genotoxic potential of 2,4-D (CCT, 1987). The lack of genotoxicity in in vitro bacterial and mammalian test system which has included an exogenous source of metabolic activation provided evidence that 2,4-D was not metabolized to potentially reactive intermediates. While in plant cells, 2,4-D has been demonstrated to induce chromosomal aberration, (Khalaktar and Bharagava, 1985; Sidorov et al., 1988, Pavilica et al., 1991). However, as discussed in the CCT report cited above, since 2,4-D was to be highly active and toxic to plants, studies on the genotoxicity of 2,4-D in this organism was thought to be more appropriate for determination of genotoxic potential.

Several studies though have been made in human population. Linnainmae (1983) and Linnainmaa and Vainio (1983) reported that there was no apparent dose response between the reported urinary 2,4-D concentration and the number of SCE's observed in peripheral lymphocytes, and SCE were elevated in both exposed group of workers who were also smokers. A similar observation was reported by Mustonen et al., (1986) in which exposure to 2,4-D had no apparent effects on SCE frequency, since the incidence of SCE was elevated in smokers of both control and exposed group. There has been several studies of human lymphocytes exposed in vivo, which indicated that the 2,4-D exposure as well as concomitant exposure to other chemicals has no effect on chromosomal aberration or SCE frequency (Hogstedr and Westerlund, 1980; Mulcahy, 1980; Linnainmaa, 1983,1984; Mustonen et al., 1986,1989). There seems to be no conclusive evidence to suggest that 2,4-D is clastogenic in humans.

The result of some SCE studies, using peripheral lymphocytes of rats, and the bone marrow of mice and Chinese hamsters indicated that 2,4-D exposure in animals resulted no significant clastogenic activity (Lamb et al., 1981; Linnainmma, 1984; Mustonen et al., 1986,1989). Two micronucleus bioassays by the exposure of 2,4-D at 100mg/Kg via intraperitoneal injection also showed no induction of micronuclei either (Jenssen and Renberg 1976; Schop et al., 1990). Nevertheless, a few researchers have reported results for in vivo genotoxicity testing of 2,4-D that would not appear to be in accordance with the weight of the experimental data (Pilinskaya, 1974; Turkula and Jalal, 1987; Adhikari and Grover, 1988). At lower doses (10 and 50mg/kg body weight) no effect on chromosomal aberrations was observed. However, the genotoxic effects did appear in rats exposed to 75, 100 and 150mg/kg for 24 h (Turkula and Jalal, 1987). Adhikari and Grover (1988) have also reported increased chromosomal damage in rat bone marrow as a result of exposure of rats to 2,4-D. Seiler (1979), reported that a single 200mg/kg body weight dose of 2,4-D induced inhibition of testicular DNA synthesis in mice. Certain other non carcinogenic agents have been observed to induce a positive response (Schop et al., 1990).

In *Drosophila melanogaster* the great majority of results have been negative (Vogel and Chandler, 1973,1974; Magnusson et al., 1977; Woodruff et al., 1983; Zimmering et al., 1985). Somatic mutations induced by 2,4-D exposure have been observed only in unstable strains of fruit fly (Magnusson et al., 1973; Rasmuson and Svahlin 1978).

Garret et al (1989), evaluated the genotoxic level of 2,4-D with a moderate response. The positive correlation between the cytogenetic damage and sperm abnormality was shown. A dose dependent increase in percentage of CA in spermatocytes and sperm head abnormalities was further reported (Amer and Aly, 2001). Reports of 2,4-D as a moderate genotoxicant also appeared simultaneously (Madrigal-Bujaidar, 2001). If some studies indicated an increase of malignant tumour in 2,4-D exposed people (Hardell, 1977), others confirmed its clastogenic effects in plant system (Ateeq et al., 2002).

Reviews of the mutagenicity of PCP were presented in NTP Technical Report 349 (NTP, 1989) and more recently by Seiler (1991). Pentachlorophenol does not appear to be a strong gene mutagen, but there is some indication that it has clastogenic potential. Results of bacterial tests for induction of gene mutations (Anderson et al., 1972; Shirasu, 1975; Simmon and Kauhanen, 1978; Haworth et al., 1983; Moriya et al., 1983) or growth inhibition due to DNA damage (Shirasu, 1975) were negative, with the exception of a study using phenobarbital- or 5,6-benzoflavone-induced rat liver S9 (Nishimura et al., 1982). However, positive results were obtained with pentachlorophenol in gene mutation assays in *Saccharomyces cerevisiae* (Fahrig, 1974; Fahrig et al., 1978) but not in assays for induction of mitotic recombination (Simmon and Kauhanen, 1978).

The results for induction of chromosome non disjunction or sex chromosome loss were negative in *Drosophila melanogaster* (Ramel and Magnusson, 1979), and further, no increase in the frequency of sex-linked recessive lethal mutations occurred in germ cells of male *D. melanogaster* (Vogel and Chandler, 1974). In cultured Chinese hamster ovary cells (CHO), pentachlorophenol (91.6% pure) induced small increases in sister chromatid

exchanges (Galloway et al., 1987), while, Ishidate and Sofuni (1985), observed no induction of chromosomal aberrations in Chinese hamster lung fibroblasts.

Additional evidence of in vitro clastogenicity came from a study in which a major rodent metabolite of Pentachlorophenol, tetrachlorohydroquinone,was shown to induce significant dose-related increases in micronuclei in CHO cells (Jansson and Jansson, 1992). Major impurities identified in the pentachlorophenol (91.6%pure), used in the NTP genetictoxicity tests were tetrachlorophenols (6.5%), octachlorodibenzop-dioxin (2%), and hexachlorobenzene (10ppm). All these compounds tested negative in the *Salmonella typhimurium* gene mutation assay (Haworth et al., 1983; Zeiger et al., 1988).

PCP is regarded as clastogenic in both in vivo and in vitro tests (Seiler, 1991), while Obe et al., (1982) suggested that in chromosomal damage, DNA is the primary target leading to misrepair or misreplication of DNA. A pointer to this mechanism could be the covalent binding of PCP to DNA. It was confirmed in an in vitro incubation with a metabolic activation system (Van Ommen et al., 1986). Rat microsomal preparation were able to convert PCP to tetachlorohydroquinone and tetrachlorocatechol in changing ratios (1.2-2.5), depending on the enzyme induction procedure used (Van Ommen et al., 1986). Both isomers were proposed to form semiquinone radicles in the presence of oxygen and this mechanism produce DNA strand breaks.

In aquatic organisms like zebra mussel and snail, PCP was able to induce micronuclei and DNA strand breaks (Pavlica et al., 2000,2002). Various forms of structural chromosomal damage in *H.fossilis was* also observed by Ali and Ahmad (1998). Recent reports on *allium* root tip showed, this chemical to be clastogenic, as it inhibited root growth and inflicted depression in mitotic index with increased frequency of CA (Ateeq at al., 2002). The micronuclei formation was shown to be due to aneugenic effect of PCP (Pavlica et al., 1999).

SUMMARY

The study aimed at evaluating the extent of genetic damage by widely used weedicides, PCP and 2,4-D by cytogenetic parameters, such as chromosome aberration (CA), micronucleus (MN) and mitotic index (MI) tests on bone marrow cells of mice, *Mus musculus* by an in vivo system.

Three sub lethal concentrations were set up according to the separate LD50 of these chemicals. Damage was noted at four different intervals of time in order to find the maximum impact of chemical on cell cycle and the functioning of its own repair system against these chemicals.

A maximum of 18.33 ± 0.05 frequency of chromosomal aberration due to PCP was recorded as against the clastogenic potential of 15.66 ± 0.05 for 2,4-D. It was seen, the maximum impact of chemical PCP on mice bone marrow was at 24 h of treatment. There was a significant rise in this duration consistently when compared to other durations.

The same pattern was observed in 2,4-D, beyond 48 h durations, clastogenic damage decreased with increase in time. Consequently, while increase in concentration, the frequency of CA likewise increased with either chemicals. No significant increase could be found in solvent and normal controls.

Both chemicals induced chromatid and chromosome aberrations, while chromatid type being more abundant. Thus chromatid type gaps were frequently observed in all concentrations of PCP and 2,4-D. Alterations such as breaks, translocations stickiness and pulverization were also observed in that order. There was an increase in stickiness and pulverization initially followed by a decline the duration increased.

Micronucleus assay authenticated these results further. The frequency of micronucleus formation showed significant increase at 24 h of treatment for both chemicals. Concentration dependent pattern was yet again confirmed. The maximum value of 1.08 ± 0.003 of MN was recorded as against the figures of 1.05 ± 0.003 in 2,4-D, a comparative mutagenic effect in favor of PCP was noticed.

An increase in micronucleated normochromatic erythrocytes was recorded at every concentration. Nonetheless it was not statistically significant when compared to the solvent and normal controls. Dose dependent increases in MNPCEs were clearly observed for PCP and 2,4-D. However no significant change in the ratio of PCEs / NCEs argues convincingly against cytotoxic effects of any of the chemicals used in present observations.

Mitotic index studies indicated an inhibition of cell division in a dose dependent manner although, the increase was not statistically significant. The mild cytotoxic effect was suggested. Observations proved the susceptibility of mammals to genetic toxicity of PCP and 2,4-D. PCP being the most toxic and 2,4-D reasonable toxic but both mutagenic mammalian system, the discontinuation or minimum possible exposure to humans is recommended therefore.

References

Adhikari N., I.-S.Grover (1988) Genotoxic effects of some systemic herbicides: In vivo chromosomal aberrations in bone marrow cells rats. Environ. Mol. Mut, 12: 235-239.

Adler, I.-D (1980) New approaches to mutagenicity studies in animals for carcinogenic and mutagenic agents, I. Modification of the heritable translocation test. Teratogen. Carcin. Mut, 1: 75-86.

*Adler, I.-D., A. El-Tarras (1989) Clastogenic effects of cis-diamminedichloroplatinum. 1. Induction of chromosomal aberrations in somatic and germinal cells of mice. Mut Res, 211: 131-137.

*Adler, I.-D., A. El-Tarras (1990) Clastogenic effects of cis-diamminedichloroplatinum. II Induction of chromosomal aberrations in primary spermatocytes and spermatogonial stem cells of mice. Mut Res, 243: 173-178.

*Adler, I.-D., G. Rohrborn (1969) Cytogenetic investigation of meiotic chromosomes of male mice after chronic caffeine treatment. Hum. Genet, 8: 81-85.

*Adler, I.-D., A. Schmaltz, R- Rathenberg, D. Mullcr, F.F. Sti-asser R. Perret (1978) Cytogenetic study in spermatocytes of mice and Chinese hamsters after treatment with isoniazid (1NH). Hum. Genet, 42: 50-54.

*Adler, I.-D., A. Neuhauser (1978) Heritable translocations induced in mouse primary spermatocytes. Mut. Res, 53: 143-144.

*Adler, I.-D (1974) Comparative cytogenetic study after treatment of mouse spermatogonia with mitomycin C. Mut. Res, 23: 369-379.

*Adler, I.-D (1966) Cytogenetic investigations of mutagenic action of caffeine in premeiotic spermiogenesis in mice. Hum. Genet, 3: 82-83.

Adler, I.-D (1976) Aberration induction by mitomycin C in early primary spermatocytes of mice. Mut. Res, 35: 247-256.

*Adler, I.-D (1982) Male germ cell cytogenetics, in: T.C. Hsu (Ed.), Cytogenetic Assays of Environmental Mutagens, Allanheld, Osmun Publishers, Totowa, NJ, pp. 249-276.

*Adler, I.-D (1990) Clastogenic effects of acrlyamide in different germ-cell stages of male mice, in: J.W. Alien, B.A- Bridges, M.F. Lyon, M.J. Moses and L.B. Russell (Eds.),

*Aeschbacher, H.U., H. Milon, H-P. Wurzner (1978) Caffeine concentrations in mice plasma and testicular tissue and the effect of caffeine on the dominant lethal test. Mut.Res. 57, 193-200.

Ahlborg, U.G., T Thunbrg (1980) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the vivo and in vitro dechlorination of pentachlorophenol. Arch. of toxicol, 40:55-61.

Ahmad. W., M.N. Ali, M. A. Farah, B. Ateeq, (2001) Computerized automated morphometric assay including frequency estimation of pentachlorophenol induced nuclear anomalies (micronucleus) in Catfish (*H. Fossils*).Chromosoma 110: 570-574.

Ali, M.N., W. Ahmad (1998) Effects of pentachlorophenol chromosomes of a catfish, *Heteropneutes fossils.* Ind. J. Exp. Biol., 36: 304-307.

Amer, S.M., F.A.E Aly (2001) Genotoxic effects of 2,4-D and its metabolites 2,4-dichlorophenol in mouse. Mut. Res., 494: 1-12.

Amer, S.M., M.E. Ali (1974) Cytological effects of pesticides on *Vicia faba,* Cytologia, 39: 633-643

*Anderson, D., M.C.E. Hodge and I.F.H. Purchase (1976) Vinyl chloride: dominant lethal studies in male Cd-1 mice. Mut. Res, 40: 359-370.

Anderson, K.J., E. G. Leighty, M.T. Takahashi (1972) Evaluation of herbicide for possible mutagenic properties. J. Agric. Food Chem. 20 : 694-656.

Anderson, B. E. Zeiger (1987) Chromosome aberration and sister chromatid exchange in Chinese Hamster Ovary Cells. Environ. Mol. Mut. 10: 1-175.

*Anderson, D., D.B. McGregor, O.F.H. Purchase, M.C.E. Hodge J.A. Cuthbert (1977) Dominant-lethal test results with known mutagens in two laboratories. Mut. Res, 43: 231-246.

*Appelgren, L.E., O. Eneroth C. Grant (1977) Studies on ethylenc oxide: whole-body autoradiography and dominant lethal test in mice. Proc. Eur. Soc. Toxicol. 18: 315-317.

Armstrong M.J., S.M. Galloway, Ashby, J (1993) 2,4,6-Trichlorophenol (TCP) induces chromosome breakage and aneuploidy in vitro. Mut. Res, 303: 101-108.

*Arnold, D.W., G.L. Kennedy Jr., M.L. Keplinger, J.C. Calandra C.J. Calo (1977) Dominant lethal studies with technical chlordane, HC'S 3260, and heptachlor: heptachlor epoxide. J.Toxicol. Environ. Health, 2: 547-555.

*Arnold, D.W., G.L. Kennedy, M.L. Keplinger J-C. Calandra (1976) Dominant lethal studies with alkalyating agents; adaose response relationship response. Toxicol.Appl. Pharmacol, 38: 79-84.

Artz. ES., A. Fernades Castdo. A Dizz. S. Finkid man, V.E. Nahanrd (1989). The muscarine agonist pilocarpine inhibits DNA and interferon Y synthesis in peripheral blood mononuclear cells. Int. J. Pharmacol. 11: 275-285.

Ashton, F.M., A.S. Crafts (1974) Mode of action of herbicides, John Wiley and Sons, New York pp. 266-288.

Ateeq, B., M.A.Farah, M.N.Ali, W.Ahmad (2002) Clastogenicity of Pentachlorophenol, 2,4-D and Butachlor evaluated by *allium root* tip test. Mut. Res 514:105-113.

*Au, W.W. T.C. Hsu (1980) The genotoxic effects of adriamycin in somatic and germinal cells of the mouse, Mutation Res. 79, 351-361, Badr, F.M, and R.S. Badr (1975) Induction of dominant lethal mutation in male mice by ethyl alcohol..Nature, 253 :134-136.

Auerbach, C (1976) Problems, Results, and perpectives London: Chapman and Hall. Mut. Res.

Aurbach, C and Robson, J.M (1946). Nature, 157- 302.

Bajpai, R., S. Banerji (1992) Bioremidation for soils contamainated with Pentachlorophenol. Annals of the New York Academy of Sciences, 665: 423-435.

*Bariliak, I.R., S.I. Kozachuk (1983) Embryotoxic and mutagenic activity of ethanol and acetaldehyde in intra-amniotic exposure. Tsitol Genet, 17: 57-60.

*Barnes, T.B., A.J. Veriangieri M.C. Wilson (1983) Reproductive toxicity of methyl-1(butylcarbamoyO-2-benzimidazole.Toxicol, 28: 103-115.

*Barnett, L.B., D.P. Lovell, C.F. Felton, BJ. Gibson, R.R. Cobb, D.S. Sharpe, M.D. Shelby I Research 341 (1994) 109-13} S.E. Lewis (1992) Ethylene dibromide:

*Basler, A. (1978) Timing of meiotic stages in oocytes of the Syrian hamster (*Mesocricetus auratus*) and analysis of induced chromosome aberrations. Hum. Genet, 42: 67-77.

Bauchinger M., J. E. Drest, R. Schmid, Hauf (1982) Chromosome changes in lymphocytes after occupational exposure to pentachlorophenol (PCP). Mut. Res, 102:83-88.

*Becker, K- J. Schoneich (1982) Expression of genetic damage induced by alkylating agents in germ cells of female mice. Mut. Res, 92: 447-464.

Bean, N. C., M. J. Armstrong , S. M. Galloway (1992). Effects of sampling time on chromosome aberration yield for 7 chemicals in Chinese hamster ovary cells.Mut. Res, 265:31-44.

*Bellies, R.P., N. Korn, B.W, Benson (1973) A comparison of the effects of methyl methane sulfonate in various reproductive toxicity screening tests. Res. Comm. Chem. Path. Pharm, 5: 713-724.

Bender, M. A., A.A Awa, A.L. Brooks (1988) Current status of cytogenetic procedures to detect and quantify previous exposure to radiation. Mut Res. 196: 103-159.

Bender, M.A., H.G. Griggs andJ.S. Bedford (1974) Mechanisms of chromosomal aberrations production. Mut.Res,13:263-273.

*Binkert, F. W. Schmid (1977) Pre-implantation embryos of Chinese hamster. 2. Incidence and type of karyotype anomalies after treatment of the paternal post-meiotic germ ceils with an alkylating mutagen. Mut. Res, 46: 77-86.

Bird, R. D., H.H. Draper, P.C. Basrur (1982). Effects of malonaldehyde and acetaldehyde on cultured mammalian cells. Production of mucnonuclei and chromosomal aberrations. Mut. Res, 101:237-246.

Black,M.C., J. R. Ferrell. R.C. Horninng, L . K. Martin Jr (1996) DNA strand breakage in fresh water mussel. (Andonla grandis) expose to lead in the lab and field. Environ. Toxocol. Chem., 5 : 802-508.

Butcher,D.N., A.K.Sogeke, .C.Tommerup (1975) Factors influencing changes in ploidy and nuclear DNA levels in cells from normal, grown, gall and habituated cultures of *Heliathus annuus* L.,Protoplasma,86:295-308

*Bleyl, D.W., R. H.J. Lcwerenz (1980) Repeated oral administration of cadmium chloride in the dominant lethal test in mice, Arch. Exp. Vet. Med. Leipzig 34:399-402.

Boberg, E.W., E.C. Miller, J.A. Miller, A. Poland, A. Liem, (1983) Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfooxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. Cancer research, 43:5163-5173.

*Bootman, J., D.C. Lodge, H.E. Whalley (1979) Mutagenic activity of propylene oxide in bacterial and mammalian systems. Mut. Res, 67:101-112.

Boveri, T (1902) The origin of malignant tumors. The William and Walkins, company.p.1

*Braun, R., E- Huttner, J. Schoneich (1986) Transplacental genetic and cytogenetic effects of alkylating agents in the mouse. II. Induction of chromosomal aberrations. Terat. Carcin. Mut, 6: 69-80.

*Brewen, J.G., H. Payne (1978) Studies on chemically induced dominant lethality III. Cytogenetic analysis of TEM-effects on maturing dictyate mouse oocytes. Mut. Res, 50: 85 92.

*Brewen, J.G., H.S. Payne, K.P. Jones, R.J. Preston (1975) Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post meiotic male germ cells. Mut. Res, 33: 239-250.

*Brusick, D., D. Matheson (1976) Mutagenic M.D. Waters et at. / Mutation evaluation of 1,1-dimcthylhydrazine, methyl hydrazine and N-phenyl-alpha-napthylamine, in: Proceedings of the 7th Annual Conference on Environmental Toxicology, pp. 108-129.

Burgdorf, W.,Kurvink,J.Cervenka (1977) Sister chromatid exchange in dyskeratosis congenitia lymphocytes. J. Med.Genet, 14: 256 –257

*Buselmaier, W., G. Rohrborn, P. Propping (1972) Mutagenicity investigations with pesticides in the host-mediated assay and the dominant lethal test in mice. Biol. Zcntralbl. 91:311-325.

*Caine, A., M.F. Lyon (1977) The induction of chromosome aberrations in mouse dictyatc oocytes by X-rays and chemical mutagens. Mut. Res, 45: 325-331.

Caligo, M.A. G. Rainaldi (1987). Origin of ara c-induced endoreduplicated cells. Mut. Res, 177: 261-265.

Carere, A.G.Cordamone,V.Ortali, M.L. Bruzzone, Di Giuseppe (1976) Mutational studies with some pesticide in *Streptomyces coelicolor* and *Salmonella thyphirium*. Mut. Res, 136

*Cattanach, B.M (1957) Induction of translocations in mice by triethylenemelamine. Nature, 180: 1364-1365.

*Cattanach, B.M. (1964) A genetic approach to the effects of radiomimetic chemicals on fertility in mice, in: W.D. Carlson and F.X. Gassner (Eds.), Effects of Ionizing Radiation on the Reproductive System, Pergamon Press, Oxford, pp. 415-426.

*Cattanach, B.M. (1966) Chemically induced mutations in mice. Mut. Res, 3: 346-353.

*Cattanach, B.M. (1967) Induction of paternal sex chromosome losses and deletions and of autosomal gene mutations by the treatment of mouse post-meiotic germ cells with triethylenemelamine. Mut. Res, 4: 73-82.

*Cattanach, B.M. (1971) Specific locus mutation in mice, in: A. Hollaender (Ed.), Chemical Mutagens: Principles and Methods for Their Detection, Plenum Press, New York-London, pp. 535-539.

*Cattanach, B.M. C.E. Pollard (1971) Mutagenicity tests with cyclohexylamine in the mouse, Mut. Res, 12:472-474.

*Cattanach, B.M. C.E. Williams (1971) A search for chromosome aberrations induced in mouse spermatogonia by chemical mutagens, Mut Res, 13: 371-375.

*Cattanach, B.M., C.E. Pollard J.H Isaacson (1968) Ethylmethane sulphonate induced chromosome breakage in the mouse . Mut. Res,6:297-307.

CCT (1987) Expert pannel reports on carcinogenicity of 2,4-D Canadian Center for Toxicology: Guelph Ontario, Canada. Chemical mutagen (thiolepa), Cytol, Genet. (USSR) 11:59-61.

Cheng, W.N., P.J. Coenraads, Z.H. Hao, G.F. Liu (1993) A health survery of workers in the pentachlorophenol section of chemical manufacturing plant. American journal of industrial medicine. 24:81-92.

Clausen M., G. Lerier, I.Witte (1990) Comparision of cytotoxicty and DNA damaging properties of 2,4-D and U46 Flund (dimethyammonium salts of 2,4-D). Arch. Toxicol, 64:497-501.

Coenraads, P.J., A. Brouwer, K. Olie, N. Tang (1994) chloracne. Some recent issues. Dermatological Clinics. 12:569-576.

Colosio, C.C., M. Maroni, W. Barcellini (1993) Toxicology and immune findings in workers exposed to pentachlorophenol (PCP). Arch. of Environ. Health. 48:81-88.

Corbett, J. R (1974) The Biochemical Mode of Action of Pesticides, Academic Press, London, New York.

Countryman, P.I., and J. A. Heedle (1976). The production of micronucli from chromosome aberrations in irridiated cultres of human lymphocytes. Mut. Res, 41: 321-332.

Crafts, A. S., W.W. Robbins (1973) Weed control, T.M.H. Edition, Tata Mc Graw Hill. New Delhi pp 660

Croker, B.H (1953) Effects of 2,4-Dichlorophenoxyacetic acid in mitosis in *Allium cepa* (bot).Gaz. 114: 274-283

Dahlhaus, M., E. Almstadt, K.E. Appeal, (1994) The pentachlorophenol metabolite tetrachoro-p-hydroquinone induces the formation of 8-hydroxy-2-deoxyguanosine in liver DNA of male B6C3F1 mice. Toxicology letters, 74:265-274.

Dalhaus.,K.E.Appeal (1995) Induction of 8-hydroxyguanosine and single strand breaks in DNA of V79 cells of tetrachloro-p-hydroquinone. Mut.Res, 329:29-36.

Darlington, C.D. (1942) Chromosome chemistry and gene action. Nature,119,66-69.

*Datta, P.K., H. Frigger and E. Schleiennacher (1970) The effect of chemical mutagens on the mitotic chromosomes of the mouse in vivo, in; F. Vogel and G. Rhorborn (Eds.), Chemical Mutagenesis in Mammals and Man, Springer Verlag, New York, pp. 194-213.

Dean,B.J., N.Danford (1984) Assay for detection of chemically induced chromosomal damage in cultured mammalian cells in:S. Vennitt and J.M.Parry (Eds)Mutagenicity Testing .A Practical Approach,IRL Press Oxford,pp-(8)-232.

*Degraeve. N., M. Chollet J. Moutschen (1984) Cytogenetic and genetic effects of subchronic treatments with organophosphorus insecticides. Arch. Toxicol, 56: 66-67

*Deknudi. G., A. Leonard, J- Arany, G. Jenar-Du Buisson and E. Delavignette (1986) In vivo studies in male mice on the mutagenic effects of inorganic arsenic. Mutagenesis 1: 33-34.

*Devi, K.R. P.P. Reddy (1980) Evaluation of Ihiotcpa for genetic damage in mice, Indian J.Exp. Bioi, 18: 866-867.

De Robertis, E.D.P., W.W. Nowinksi, D.A. Saez (1974) General Cytology, Saunders, London.

*Dean B.J., D.Blair (1976) Dominant lethal assay in female mice after oral dosing with dichlorvos or exposure to atmospheres containing dichlorvos. Mut. Res, 40: 67-72.

*Dean, B.J., E. Thorpe (1972) Studies with dichlorvos vapour in dominant lethal mutation tests on mice. Arch. Toxicol, 30: 51-59.

*Dean, B.J., A. Johnstone (1977) Dominant lethal assays in male mice; evaluation of experimental design, statistical methods and the sensitivity of Charles River (CD1) mice. Mut. Res, 42: 269-278.

*Dean, B.J., S.M.A. Doakand H. Somerville (1975) Potential mutagenicity of dieldrin (HEDD) in mammals. Food Cosmet, Toxicol, 13: 317-323.

Derscheid,L.A.,L.N.StahlerD.E Kratochvil (1952) Differential responses of barley Varities to 2,4-D, Agron.J.,44,182-188.

*Degraeve, N. J. Moutschen (1984) Absence of genetic and cytogenetic effects in mice 122 M.D. Waters e! at. / Mululiun treated by the organophosphorus insecticide parathion, its methyl analogue, and paraoxon.Toxicol, 32: 177-183.

Demerec, M (1956) Carnegie Institute of Washington Publ, 612.

Denton, T.E., W.F.Howell (1969) Copeia.2:392.

Dev, K (1973) Effects of some herbicides on the structures and behaviour of plant chromosomes. Proc Indian Sci. cong, 60: 308.

*Dobrzynska, M., M. Lenarczyk and A.K. Gajewski (1900) Induction of dominant lethal mutations by combined X-ray-acrylamide treatment in male mice. Mut. Res, 232, 209-215.

Dolls (1994) Toxicology : The basic science of poisons. Fifth edition,McGraw Hill*

EEC (1979). Methods for detection of toxicology, EEC directive 79/831, Official Journal of the European communities No. 2259/10.

*Ehling, U.H. (1977) Dominant lethal mutations in male mice, Arch. Toxicol, 38: 1-11.

*Ehling, U.H. (1971) Comparison of radiation- and chemically-induced dominant lethal mutations in male mice. Mut. Res, 11: 35-44.

*Ehling, U.H. (1974) Differential spermatogenic response of mice to the induction of mutations by antineoplasic drugs. Mut. Res, 26: 285 295.

*Ehling, U.H. (1978) Specific-locus mutations in mice, in: A. Hollaender F.J. de Serres (Eds.). Chemical Mutagens: Principles and Methods for Their Detection, Plenum Press, New York-London, pp. 233-256.

*Ehling, U.H. A. Neuhauser (1979) Procarbazinc-induced specific locus mutations in male mice. Mut. Res, 59: 245-256.

*Ehling,U.J.H. A. Neuhauser-Klaus (1988) Induction of specific-locus and dominant lethal n Research 34! (1994) 109-131 mutations in male mice by diethyl sulfate (DES). Mut. Res, 199: 191-198.

*Ehling, U.H. A. Neuhauser-Klaus (1984) Dose-effect relationships of germ-cell mutations in mice, in: Y. Tazima, S. Kondo and Y.

*Ehling, U.H. A. Neuhauser-Klaus (1989) Induction of specific-locus mutations in male mice by ethyl methanesulfonate (EMS), Mut. Res. 227, 91-95.

*Ehling, U.H. A. Neuhauser-Klaus (199la) Induction of specific-locus and dominant lethal mutations in male mice by busulfan. Mut. Res, 249:285-292.

*Ehling, U.H. A. Neuhauser-Klaus (199lb)Induction of specific-locus and dominant lethal mutations in male mice by 1-methyl-l-nitrosourea (MNU). Mut. Res, 250, 447-

*Ehling, U.H., A. Neuhauser-Klaus (1992) Re evaluation of the induction of specific-locus mutations in spermatogonia of the mouse by acrylamide. Mut. Res, 283:185-191.

*Ehling, U.H., D.G. Doherty, H.V. Mailing p (1972) Differential spermatogenic response of mice to the induction of dominant-lethal mutations by n-propyi methanesulfonate and iso pi propyl methanesulfonate. Mut. Res, 15, 175-184.

*Ehling, U.H., R.B. Cumming, H.V. Mailing (1968) Induction of dominant lethal mutations by alkylating agents in male mice. Mut. Res, 5: 417-428.

*Eibs, H.G., H. Spielmann (1977) Inhibition of F(post-implantation development of mouse biastocysts in vitro after cyclophosphamide treatment in vivo. Nature (London) 270: 54-56. pr

*Embree, J.W., J.P. Lyon, C.H. Hine (1977) The mutagenic potential of ethylene oxide using the dominant-lethal assay in rats. Toxicol. Pr Appl. Pharmacol, 40: 261-267.

Environmental Protection Agency-Federal Insecticide, Fungicide and Rodenticide Act; Good Laboratory Practice Standard, Federal Register, 40 CFR Part 160, 1990.

Environmental Protection Agency-Pesticide Assessment guideline (1984) Subdivision F, Hazard Evaluation: Human and Domestic Animals. Series 82-1,

83-1,and 83-2, US Department of Commerce, National Technical Information Service PB86-108958, Revised Edition, November.

Environmental Protection Agency-Pesticide Assessment Guidelines, Subdivison F. Hazard Evaluation: Human and Domestic Animals, Series 81, 82, and 83, US Department of Commerce. National Technical Information Service PB91-154617, 1991.

EPA (1952) Heath effects test guidelines (TSCA), Environmental Protections Agency Report. EPA 560/6-82001.

*Epstein, S.S. and H. Shafner (1968) Chemical mutagens in the human environment, Nature (London). 219: 385-387.

*Epstein, S.S., E. Arnold, J. Andrea, W. Bass and Y. Bishop (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol, 23: 288 25.

*Epstein, S.S., E. Arnold, K. Steinberg, D. Mack pr' intosh, H. Shafner and Y. Bishop (1970) Muta, genie and antifertility effects of TEPA and, METEPA in mice. Toxicoi. Appl. Pharmacol, 17: 23-40.Ga,

*Epstein, S.S., W. Bass, E. Arnold, Y. Bishop (1970b) Mutagenicity of trimethylphosphate in mice. Science, 168: 584-586.

*Epstein, S.S., W. Bass, E. Arnold, Y. Bishop, S. Joshi and I.-D. Adier (1971) Sterility andsemisterlity in male progeny of male mice treated with the chemical mutagen TCPA.Toxicol. Appl.Pharmacol 19: 134-146.

*Epstein. S.S., W. Bass, E, Arnold Y. Bishop (1970a) The failure of caffeine to induce muta fvf.D. Waters et ai / Mutation I genie effects or to synergize the effects of known mutagens in mice. Food Cosmet. Toxicol, 8: 381-401.

European Economic Community (1988), Methods for the determination of toxicity. Official Journal of the European Communities L133 .

European Economic Community, Good Laboratory Practice, Official Journal of European Communities L15 (1987).

Evans, H. J., G. J. Neary, F. S. Williamson (1959). The relative ecological efficiency of single doses of fast neurons and gamma rays *on vicia faba* roots and the effect of oxygen. II Chromosome damage : The production of micronucleus Int. J. Radat, Biol., 3, 46-229.

Exon, J.H., L.D. Koller (1982) Effects of transplacental exposure to chlorinated phenols. Environmental health perspectives, 46:137-140.

Exon, J.H., L.D. Koller (1983) Effects of chlorinated phenols on immunity in rats. Intern. Journ. of immunopharm, 5: 131-136.

Fahrig, R (1974) Comparative mutagenicity studies with pesticides. IARC Sci. Publ. 10: 161-181.

Fartington, M. A.J. Bateman (1964) Dominant lethal mutations induced in male mice by methyl methanesulphonate Heredity, 19: 191-200.

Fahrig, R. (1974). Comparative mutgenicity studies with pesticides IARC Sci. Publ. 10: 161-181.

Farah. M. A., B. Ateeq, M. N. Ali, W. Ahmad (2001) Evaluation of Genotoxicity of PCP and 2,4-D by micronucleus test in fresh water fish *Channa punctatus*. Mut. Res. 54: 25-29.

Farig, R, C.A. Nilsson, C. Rappe (1978) Genetic activity of Chlorophenol and chlorophenol impurities. In Pentachlorophenol: Chemistry, Pharmacology and Environ. Toxocology (K. R. Rao Ed.) pp. 325-338. Plenum Press, New York.

*Favor, J. (1986) The frequency of dominant cataract and recessive specific-locus mutations in mice derived from 80 or 160 mg ethyinitrosourea per kg body weight treated spermatogonia. Mut. Res, 162: 69-80.

*Favor, J. J.W. Crenshaw (1978) EMS-induced dominant lethal dose response curve in DBA/1J male mice. Mut. Res 53: 21-27.

Fedral Register (1990) Volume 55, pp 115. 24116. EPA Proposed Rules.
The induction of heritable gene mutations by dibromochloropropane (DBCP). Mut Res, 170: 161-166.

Fiscor, G., G.M.Wiilo Piccolo (1972) Survey of pesticides for mutagenicity by the bacterial plate assay method. Ems Newsletter, 6: 6-8.

*Fonatsch, C. (1977) Effect of nitrofurantoin on meiosis of the male mouse. Hum, Genet, 39: 345-351.

*Fontignie-Houbrechts, N. (1981) Genetic effects of formaldehyde in the mouse. Mut Res. 88:109-114.

Forest Service, (1984). Pesticide Background Statements, Vol. I Herbicides. United States Department of Agriculture, Agriculture Handbook No. 633.

*Fox, M. D. Scott (1980) The genetic toxicology of nitrogen and sulphur mustard, Mutation Res. 75, 131-168.

*Franz, J. (1978) Mutagenicity of isoniazid: testing for somatic chromosome aberrations in mouse embryos. Hum. Genet, 42: 28-30.

*Friedman, L., H.L. Richardson, M.E. Richardson, E.J. Lethco, W.C. Wallace and F.M. Saura (1972) Toxic response of rats to cyclamates in chow and semisynthetic diets, J. Natl. Cancer Inst, 49: 751-764.

*Fritz, H., D. Muller R. Hess (1973) Embryo lethality in the mouse following treatment of males with cyclophosphamide at specific germ cell stages. Agents Actions 3: 35-37.

Galloway, S. M., Armstrong, M. J., Reuben, C., Colamn, S. Brown, B. Canmon, C. Bloom, A. D. Nakmura F. Ahmed, M. Duk. S. Rimpo, J. Murgolion, B.H. Resnick, M. A. Haworth, S., T.Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983) Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. 5: 3-142.

Galloway, S.M M.J Armstrong, C Renbens, S.Colman B.Brown, C. Cannon, J.Rimpo.B.H Margolin, M.A Resnick B. Anderson, Zeiger (1987) Chromosome aberration and sister chromosome exchanges in Chinese Hamster Ovary cells. Evaluation of 108 chemicals, Environ. Mol Mut. 10:1-175.

Garret, N.E., H.F.Stack M.D. Waters (1989) Evalution of the genetic acitivity of 65 pesticides. Mut.Res, 163:301-305.

*Garthoff, L.H., L. Friedman, T.M. Farber, K.K. Locke. T.J. Sobotka, S. Green, N.E. Hurley, E.L. Peters, G.E. Story, F.M. Moreland, C.H. Graham, J.E. Keys, M.J, Taylor, J.V. Scalera, J.E. Rothkin, E.M. Marks, F.E. Cerra, S.B. Rodi and E.M. Sporn (1977) Biochemical and cytogenetic effects in rats caused by short-term ingestion of aroclor 1254 or firemaster BP6. J. Toxicol. Environ. Health 3: 769-796.

*Generoso, W.M, K.T. Cain, C.S. Hellwig and N.L.A. Cacheiro (1982) Lack of association between induction of dominant-lethal mutations and induction of heritable translocations with benzo (a] pyrene in postmeiotic germ cells of male mice. Mut. Res, 941:55-163.

*Generoso, W.M-, K.T. Cain, L.A. Hughes, G.A. Sega, P.W. Braden, D.G-Gosslee and M.D. Shelby (1986) Ethylene oxide dose and dose rate effects in the mouse dominant-lethal test. Environ. Mol. Mut. 8: 1-7.

*Generoso, W.M. W.L. Russell (1969) Strain and sex variations in the sensitivity of mice to dominant-lethal induction with ethyl methane sulfonate. Mut. Res, 8: 589-598.

*Generoso, W.M., K.T. Cain, L.A. Hughes (1985) Tests for dominant-lethal effects of 1,2,dibromo-3-chloropropane (DBC male and female mice), Mut.Res, 156:103-108.

*Generoso, W.M., K.T. Cain, C.C. Cornett, N.L.A. Cacheiro (1984) DNA target sites associated with chemical induction of dominant lethal mutations and heritable translocations in mice. in: V.L. Chopra et al. (Eds.), Genetics: New Frontiers, Oxford & IBH Publ. Co., New Delhi, pp. 347-356.

*Generoso, W.M., K.T. Cain, C.V. Cornett, N.L.A. Cacheiro and L.A. Hughes (1990) Concentration-response curves for ethylene-oxide-induced heritable translocations and dominant lethal mutations. Environ. Mol. Mut, 16: 126-131.

*Generoso, W.M., K.T. Cain, L.A. Hughes and L.B. Foxworth (1989) A restudy of the efficacy of adriamycin in inducing dominant lethals in mouse spermatogonia stem cells. Mut. Res, 226: 61-64.

*Generoso, W.M., K.T. Cain, M. Krishna, C.W. Sheu and R.M. Gryder (1980) Heritable tranlocation and dominant lethal mutation. Induction with ethyleneoxide in mice. Mut.Res, 73:133-142.

*Generoso, W.M., K.T. Cain, S.W. Huff, D.G. Gosslee (1978) Inducibility by chemical mutagens of heritable translocations in male and female germ cells of mice. Advan In Modern Toxicol, 5: 109-129.

*Generoso, W.M., M, Krishna. R.E. Sotomayor, N.L.A. Cacheiro (1977) Delayed formation of chromosome aberrations in mouse pachytene spermatocytes treated with triethyienemelamine (TEM). Genetics, 85: 65-72.

*Generoso, W.M., R.B- Cumming, J.A. Bandy, T. Cain (1983) Increased dominant-lethal effects due to prolonged exposure of mice to inhaled ethylene oxide. Mut. Res, 119: 377-379.

*Generoso, W.M., R.J. Preston, J.G. Brewen (1975) 6-Mercaptopurine, an inducer of cytogenetic and dominant-lethal effects in premeiotic and early meiotic germ cells of male. Mut. Res, 28: 437-447.

*Generoso, W.M., S.W. Huff, S.K. Stout (1971) Chemically induced dominant-lethal mutations and cell killing in mouse oocytes in the advanced stages of follicular development. Mut. Res. 11: 411-420.

*Generoso, W.M., S.W. Huff, K.T. Cain (1975) Comparative inducibility by 6-mercaptopurine of dominant lethal mutations and heritable translocations in early meiotic male germ cells and differentiating spermatogonia of mice. Mut. Res, 31: 341-342.

*Generoso, W.M., W.L. Russell. S.W. Huff, S.K. Stout, D.G. Gosslee (1974) Effects of dose on the induction of dominant-lethal mutations and heritable translocations with ethyl methanesulfonate in male mice. Genetics, 77: 741-752.

*Generoso. W.M., S.W. Huff, K.T. Cain (1979) Relative rates at which dominant-lethal mutations and heritable translocations are induced 124 A/. /? Wo^/-? et al. / Mutation by alkylating chemicals in postmeiotic male germ cells of mice. *Genetics* 93: 163-171.

*Gilliavod, N. A. Leonard (1971) Tests for mutagenic effects of chemicals in mice. I. Effects of mitomycin C on spermatogonia, Mutation Res. 13, 274-275.

*Gilliavod, N. and A. Leonard (1975) Mutagenicity tests with cadmium in the mouse. Toxicol, 5: 43-47.

Giri A. C., L. Banerjee, C. Talukder, A. Sharma (1981). Chromosomal and cellular changes induced by organo phosphorus insecticdes, in. C. R. Manna and U. Sinha (Eds.), Perspectives in Cytology and Genetics, Vol. 5, pp. 407-410.

*Goetz, P., A.M. Malashenko, N.I. Surkova (1980b) Chromosome aberrations induced by cyclophosph amide in meiotic cells of male mice. Tsitol. Genet, 14: 29-35.

Goldstein, J.A., M. Friesen, R.E Linder. (1977) Effects of pentachlorophenol on hepatic drug-metabolizing enzymes and porphyria related to contamination with chlorinated dibenzo-p-dioxins and dibenzofurans. Biochemical pharmacology, 26: 1549-1557.

Grant, W.F (1973) Cytological effects of environmental mutagens –pesticides. Mut. Res, 21: 221-222.

*Green, S., F.M. Moreland, W.G. Flamm (1977) A new approach to dominant lethal

*Green, S., J.V. Carr, F.M- Sauro and M.S. Leator (1973) Effects of hycanthone on spermato gonial cells, deoxyribonucleic acid synthesis in bone marrow and dominant lethality in rats. J.Pharmacol. Exp. Ther, 187: 437-443.

*Green, S., P.M. Sauro and L. Friedman (1975) Lack of dominant lethality in rats treated with polychlorinated biphenyls (aroclors 1242 and 1254), Food Cosmet. Toxicol, 13: 507-510.

Gurr, J. R., Y. C Lies, I.C. Ho, E. Y. Jan, T.C. Lee (1993). Induction of chromatic breaks and tetraploidy in Chinese hamster Ovary cells by treatment with sodium arsenite, during 62 phase. Mut. Res, 319: 135-142.

Hakeem, H., S. Amer (1965). Meiotic effects of water extracts of some contraceptive tablets, Naturwissen Schaften, 53,184.

*Halkka, 0., K. Eriksson (1977) The effects of chronic ethanol consumption on goniomitosis in the rat, Adv. Exp. Med. Biol, 85:1-6.

*Hansmann, I (1974) Chromosome aberrations in mctaphase II-oocytes stage sensitivity in the mouse oogenesis to amethopterin and cyclophosphamide. Mut. Res, 22 175-191.

*Hansmann, I (1973) Induced chromosomal aberrations in pronuclei, 2-cell stages and morulae of mice. Mut. Res, 20: 353-367.

Harshabarger, M.A., J.B Clark (1990) Epizootiology of neoplasm in bony fish of north America, Sci Total Environmental, 94:1-32.

*Hardin, B.D., R.L. Schuler, P.M. McGinnis, R.W. Niemeier and R.J. Smith (1983) Evaluation of propylene oxide for mutagenic activity in 3 in vivo test systems. Mut. Res. 117: 337-344.

Hartman, P.E., Hartman, Z, Stahl, R.C. Ames, B.N (1971) Adv. Genet.161.

Hardell, L (1977) Malignant mesenchymal tumors and exposures to phenoxy acids, a clinical observation. Lakartidningen, 74 2753-2754. (Swedish)

*Hastings, S.E., K.W. Huffman and M.A. –Gallo (1976) The dominant lethal effect of dietary triethylenemelamine. Mut. Res, 40: 371-378.

Haworth, S.,T Lawlor,K Mortelmans, W. Speck, E.Zeiger (1983) Salomonella mutagenicity test result for 250 chemicals. Environ.Mut,5:3-142

Heedle, J. A., A.V. Carrano (1977). The DNA content of nicronuclei induced in mouse bone marrow by gamma-irridiations. Evidences that micronuclei arise from accentric chromosomal fragments. Mut. Res, 44: 63-69.

Hedge,M.J.T.V.Sujatha (1995) In vivo genotoxicity of the alkaloid drug Pilocarpine nitrate in bone marrow and male germ cell of mice.Mut.Res, 344:103-108.

*Herbold, B.A., L. Machemer, G. Rohrborn (1982) Mutagenicity studies with 2,4,5-T on bacteria and mammalian germ cells. Terat. Carcin. Mut. 2: 91-101-

*Himeno. S., H. Okuda, T. Suzuki (1983) Lack of dominant lethal effects in male CD-1 mice after short-term and long-term exposures to vinyl chloride monomer Toxicol. Lett, 16: 4753.

*Hitotsumachi, S. and Y. Kikuchi (1977) Chromosome aberrations and dominant lethality mouse embryos after paternal treatment with triethylenemelamine. Mut. Res, 42: 117-124.

Hoar, S. K., A. Blair, F.F.Holmes, C.D.Boysen, R.J.Robel, R. Hoover, J.F. Fraumeni , Jr (1986) Agricultural Herbicide use and Risk of Lymphoma and Soft –Tissue Sarcoma, JAMA 256: 9:1141-1147.

Hoffman,G.R (1996) Genetic Toxicology In : Casarret and Doull's Toxicology: The basic science of poisons, fifth edition, Mc Graw Hills*

Hogsterd C., B.Westerlund (1980) Cohort studies of the cause of death of forestry workers with and witout exposure to phenoxy acids preparation.Lakartidninges, 77: 1828-1831.

Hsu, T.C (1952) Mammalian chromosome in vitro I. The Karyotype of man J. Haredita. 43 : 167-172.

IARC (1986) Some halogenated hydrocarbons and pesticide exposure. Lyons, International Agency For Research on cancer, pp 319-356(IARC monographs on the Evaluation of the carcinogenic Risks to Humans, Vol.53.)

IARC (1982) Chemicals, industrial processes and industries associated with cancer in humans, IARC Monographs, Volumes 1 to 29. Lyon, International Agency for Research and Cancer, pp. 249-250 (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Suppl. 4).

IARC (1986) Monographs on the evaluation of, Carcinogenic Risk of Chemicals to Humans, Vol.41, IARC Lyon, pp. 357-406. 21 Forest Service, (1984) Pesticide Backgroung Statement, Vol. I Herbicides, United States, Department of Agriculture, Agricultural Handbook No. 633.

IARC (1991) Occupational exposures in insecticide application, and some pesticides. Lyon, International Agency for Research on Cancer, pp. 371-402 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 53).

Innes, J.R., B.M. Ulland, M.G. Valerio, L. Petruculli, L. Fishbein, E.R. Hart, A.J. Pallotta, B.R. Bates (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. Journ. of the National Canc. Inst, 42:1101-1114.

Ishidate, J. R. T. Sofuni (1985) The in vitro chromosomal aberration test using Chinese Hamster Lung CHL (fibroblast cells in culture). In progress in Mutaion Research . Evaluation of short term tests for Carcinogenicity J. Ashby, F. J. de Serres, M. Draper, M. Ishidiate, Jr. , B. H. Margolin, B.E. Matler, M. D. Shelby Eds. Vol. 5 pp. 427-432.Elsevier Science Publishers Amsterdams.

Jagetia,G.C., P.S. Jacob (1998) Vinablastine treatment induces dose dependent increase in the frequency of micronuclei in mouse bone marrow. Mut. Res. 280: 8, 7-92.

Jansson, K., V. Jansson (1992) Induction of micronuclei in V79 Chinese Hamster Cells by tetrachlorohydroquinone, a metabolite of pentachlorophenol . Mut. Res. 279: 205-208.

Jacobi H., J. Witte (1991) Synergistic effects of U46D Flund (dimethylammonium salts of 2,4-D) and CuCl2 on cytotoxicity and DNA repair in human fibroblast .Toxicol Letters 58:159-167.

*James, D.A. and D.M. Smith (1982) Analysis of results from a collaborative study of the dominant lethal assay. Mut. Res, 97: 303-314.

Jansson, K., V, Jansson (1992b) Genotoxicity of 2,4,6-trichlorophenol in V79 Chinese hamster cells. Mut. Res, 280: 175-179.

Jansson, K., V. Jansson (1992a) Induction of micronuclei in V79 Chinese hamster cells by tetrachlorohydroquinone, a metabolite of pentachlorophenol. Mut. Res, 279:205-208.

Jenssen, D., and C. Ramel (1976) Dose response at low doses of X-irradiation and MMS on the induction of micronuclei in mouse erythroblasts. Mut. Res, 41:311-320.

Jenssen, D., L.Rengerg (1976) Distribution and cytogenetic tests of 2, 4-D and 2,4,5-T phenoxyacetic acids in mouse blood tissues. Chem. Biol Interact 14: 291-299.

Johnson, E.S (1990) Association between soft tissue sarcomas, malignant lymphomas, and phenoxy herbicides/chlorophenols: evidence from occupational cohort studies. Fund and App.l Toxicol, 14:219-234.

Jorens, P.G., P.J. Schepens, (1993) Human pentachlorophenol poisoning. Hum and Experim. Toxicol 12:479-495.

Juli, Y., Y. Nagae, T. Ishikaua. Y. Watanabe. T.N.A. Gashima, IC Matsukubo H. Shimizu (1969). Effect so erythropoietin on the micronucleus test. Envivon. Mol. Mut. 13:314-318.

Kabarity, A., S. Mazrooei (1984). Further investigations of the cytological effects of some contraceptives. Mut. Res., 135,181-188.

Kallak, H., L, Jarrekylg (1971) Cytogenetic effects of 2,4-D on pea callus in culture. Acta Biol.Budapest, 22: 67-73

*Kapp, R-W., Jr. (1979) Mutagenicity of 1,2-dibromo-3-chloropropane (DBCP); in vivo cytogenetics study in the rat, Toxicol. Appl. Pharmacol, 48, A46.

Kar.S (1975) Cytogenetic effect of 2,4-D on *Pisum sativum,* (Abstract no.234)Proc.Indian Sci .Congr. (Bot.),62:122.

*Kennedy, G.L., Jr., D.W. Arnold M.L,Keplingur (1975) Mutagenicity studies with captan, captofol, folpet and thalidomide. Food Cosmet. Toxicol, 13: 55-61.

Kerkvliet, N.I., L Schmitz (1882) Immunotoxicity to technical pentachlorophenol (PCP) Depressed humoral response to T- dependent and Tindependent antigen stimulation in PCP-T exposed mice, Fundamentals and Applied Toxocology 2:90-99.

Kerkvliet, N.I., Baecher-Steppan, J.A.Schmitz (1982) Immunotoxicity of Pentachlorophenol (PCP):increased susceptibility to tumor growth in adult mice fed technical PCP contaminated diets. Toxicology and Applied Pharamacology 62:55-64.

Khalarkhare A.S., V.R. Bhargava (1985) Mutagenic effects of 2,4-D in rats. J.Agr. Food Chem .4: 500-503

*Khera, K.S. (1974) Teratogenicity and dominant lethal studies on hexachlorobenzene in rats, Food Cosmet. Toxicol, 12:471-477.

Kimbrough R.D, R.E. Linder (1978) The effect of technical and purified pentachlorophenol on the rat liver. Toxicol. and Appl pharmacol, 46:151-162.

*Klassen. R.W. T.V.N. Persaud (1976) Experimental studies on the influence of male alcoholism on pregnancy and progeny. Exp. Pathol, 12: 38-45.

*Kohila, T., K. Eriksson and 0. Halkka (1976) Goniomitosis in rats subjected to ethanol. Med. Biol, 54: 150-151.

Koistinen J Paasivirta, J. Suonpera M. Hyvarinen.H (1995) Contamination of pike and sediment from the Kymijoki river by PCDEs, PCDDs, and PCDFs: contents and patterns compared to pike and sediment from the Bothnian Bay and seals from Lake Saimaa. Environ Sci and Tech, 29:2541-2547.

Korte, C., S.M.Jalal (1982) 2,4-dichlorophenoxyacetic acid induced clastogenicity and elevated rates of sister chromatid exchanges in cultured Human lymphocytes. J Heredity, 73:224-226.

*Kozachuk. S.Y., I.R. Barilyak 1982) Ultrastructure and histochemical characteristics of embryonic hepatocytes under the action of ethanol. Tsitol. Genet, 16: 59-68.

Kramers, P.G.N., A.G.A.C. Knaap, C.A. Vander Heijden. R.D.F.M. Taalman G.R. Mohn (1991). Role of genotoxicity assays in the regulation of chemicals in the Netherlands. Considerations and experience. Mutagenesis, 6: 487-493.

*Kuhlmann, W., H.-G. Fromme, E.M. Heege W. Ostertag (1968) The mutagenic action of caffeine in higher organisms. Cancer Res, 28: 2375-2389.

Laine, M.M., K.S. Jorgensens (1996) Straw compost and Bioremediated oil as inocla for the bioremediation of chlorophenol contaminated soil. Appl, Environ Microb. 62: 1507-1513

Lambert, J. Magnusson (Eds.) (1981) Genetic Toxicology of Environmental Chemicals, Part

Lamb, J.C, T.A.Marks, B.C. Gladen, J.W.Aleen, J.A.Moore (1981) Male fertility sister chromatid exchange, and germ cell toxicity following exposures to mixutures of chloriated phenoxy acids containing 2.3,7,8- tetrachlorodibenzo-p-dioxin.J.Toxicol.Environ .Health 8:825-834.

Lampi P T. Hakulinen, T. Luostarinen, Epukkala, L. Teppo (1993) Long-term chlorophenol exposure causes gastrointestional and sin symptoms in humans. In: Proceedings of the first Finnish conference of environmental sciences. Kuopio, Kuopio University, pp. 412-415 (Kuopio University Publication 14).

*Lane, R.W., G.S. Simon, R.W. Dougherty, J.L. Egle Jr. J.F. Borzelleca (1985) Reproductive toxicity and lack of dominant lethal effects of 2,4-dinitrotoluene in the male rat. Drug Chem. Toxicol, 8: 265-280.

*Lang, R. and I.-D. Adler (1977) Heritable translocation test and dominant-lethal assay in male mice with methyl metanesulphonate. Mut. Res. 48:75-88.

*Legator, M.S., K.A. Palmer, S. Green K.W. Petersen (1969) Cytogenetic studies in rats of cyclohexylamine, a metabolite of cyclamate, Science, 165: 1139-1140.

*Leonard, A. G. Linden (1972a) Mutagcnic properties of cyclamates in mammals. C.R.Seances Soc. Biol. Ses Fil, 166 468-470.

*Leonard, A. G. Linden (1972b) Observation of dividing spermatocytes for chromosome aberrations induced in mouse spermatogonia by chemical mutagens. Mut. Res. 16, 297-300.]

Lerda, D., R.Rizzi (1991) Study of reproductive functions in persons ocupationally exposed to 2,4-D Mut.Res,262:47-50.

*Levine, B.S., M.M. Preache E. Pergament (1980) Mutagenic potential of cis-dichlorodiammine platinun: II in rodents, Toxicol, 17: 57-65.

*Lewis, S.E., L.B. B-rnett, C. Felton, P.M. Johnsom, L.C. Skow. N. Cacheiro M.D. Shelby (1986) Dominant visible and clectrophoretically expressed mutations induced in male mice exposed to ethylene oxide by inhalation, Environ. Mol. Mut. 8: 867-872.

Lianainmaa, K (1984) Induction of sister chromatid exchanges by the peroxisomes proliferators 2,4-D,MCPA and Clofibrate in vivo and in vitro. Carcinogenisis 5: 703-707.

Lianainmaa K., H.A.D.Vanio (1983) SCE's among herbicide sprayers exposed to 2, 4 –D and MCP (1983). Mut. Res, 113:276-277.

Linnainmae, K (1983) Sister chromatid exchanges among workers occupationally exposed to phenoxyacid herbicides, 2,4-D and MCPA.Trat.Carcin. Mut.Res 3: 269-279.

Libbenga, K.R., J.G.Torrey (1973) Hormone induced endo reduplication prior to mitosis in cultured pea root cortex cells. Am. J. Bot, 60: 293-29

Lilienfeld DE, Gallo MA (1989) 2,4-D, 2,5-T, and 2,3,7,8-TCDD : An overview. Epidemiological review 11:28-58.

Loos, N.A (1975) Phenoxy alkanoic acids, in: P.C.Kearney and D.D.Kaufman (EDS.),Herbicides.Chemistry , Degradation and Mode of Action, (ed),Marcel Dekker. New York.pp.1-128.

*Lorke, D. (1973) Investigation of cyclamate for mutagenic effects by use of the dominant lethal assay in the mouse. Hum.Genetik 18: 165-170.

*Lorke, D. L. Machemer (1975) Influence of several weeks' treatment of male and female mice with saccharin, cyclamate or cyclohexyiamine sulfate on fertility and dominant lethal effects. Hum Genetik ,26: 199-205.

*Luippold, H.E., P.C. Gooch J.G. Brewen (1978) The production of chromosome aberrations in various mammalian cells by triethylenemelamine, Generics, 88: 317-326.

*Lyon, M.F., R.J-S. Phillips A.G. Searle (1962) A test for mutagenicity of caffeine in mice, Z. Vererbungsl. 93: ?-13,

*Machemer, L. D. Lorke (1976) Evaluation of the mutagenic potential of cyclohexylamine on spermatogonia of the Chinese hamster. Mut. Res, 40: 243-250.

*Machemer, L. D. Lorke (1975b) Experiences with the dominant lethal test in female mice: effects of alkylating agents and artificial sweet126 M.D. Waiers et ul. / Mutation oners on pre-ovulatory oocyte stages. Mut. Res. 29: 209-214.

*Machemer, L. D, Lorke (1975a) Method for testing mutagenic effects of chemicals on spermatogonia of the Chinese hamster, Arzneim. Forsch, 25: 1889-1896.

*Machemer, L. R. Hess (1971) Comparative dominant lethal studies with phenyibutazone, thio-tepa and MMS in the mouse. Experientia, 27: 1050-1052.

McMahon, R.M., E.R.Whicus , C.A. Berger (1960) Some cytological characteristics of 2,4-D induced mitosis in polyploidy cells of *Solanum-Tuberosum*,Caryologia,12:398-403.

Madrigal-Bujardar, E.A. Harnandes –Ceruelos, G.Chamoro (2001) Induction of sister chromatid exchanges by 2,4-dichlorophenoxyacetic acidin various rainbow trout in 4th congress of toxocology in developing countries Antalay Turkey.

Magsusson I., C.Ramel, A.Eriksson (1977) Mutagenic effects of chromatid phenoxy acetic acids in *drosophila melanogaster* Hereditas , 87: 121-123.

*Maier, P. G. Zbinden (1980) Specific locus mutations induced in somatic cells of rats by orally and parenterally administered procarbazine, Science, 209: 299-301.

Maier, H .Bode, (1971) Herbizide and ihre, Ruckstande,Ulmer, Stuttgart, pp. 62-113.

*Mailhes, J.B., (1983) Methylmercury effects on Syrian hamster metaphase II oocyte chromosomes, Environ. Mol. Mut. 5, 679-686

Mailhes. J.B., M.J. Aardema (1992) Benomyl-induced aneuploidy in mouse oocytes, Mutagenesis, 7:303-309.

*Malashenko, A.M., N.I. Surkova (1974) Mutagenic effect of thio-tepa in laboratory mice. I. chromosome aberrations in somatic and germ cells of male mice. Sov. Genet, 10:51-58.

*Malashenko, A.M., P. Goetz (1981) Cytogenetic analysis of translocations induced by the chemical mutagcn thiotepa in spermatids of male mice. Folia Biol. (Prague), 27: 178-185.

*Malashenko, A.M., T.B. Beskova (1988) Induction of chromosome defects by thiophos phamide in spcrmatogonia of mice of inbred lines 101HY, PTS/Y and CBA/LacY, Sov. Genet, 24:320-325.

*Malashenko, A.M., K.K. Semenov, G.P. Selezneva and N.I. Surkova (1978) Investigationmale mice with methyl methanesulfonate. Mut .Res, 48: 75-88.

*Mankes, R.F., R, Lefevre, K.-F. Benitz, I. Rosenblum, H. Bates, A.I.T. Walker and R. Abraham (1982) Paternal effects of ethanol in the Long-Evans rat, J. Toxicol. Environ. Health, 10: 871-878.

*Manyak, A., E. Schleiermacher (1973) Action of mitomycin C on mouse spermatogonia. Mut. Res, 19: 99-108.

*Matter, B.E. (1982) Heritable translocation test in mice with triethylene-melamme (TEM) and ergotamine. Mut. Res, 104: 177-182,

*Matter, B.E. I, Jaeger (1975) Premature chromosome condensation, structural chromo some aberrations, and micronuclei in early mouse embryos after treatment of paternal postmeiotic germ cells with triethyleneme lamine possible mechansisms for chemically induced dominant-lethal mutations. Mut. Res, 33: 251-260.

*Matter, B.E. W.M, Generoso (1974) Effects of dose on the induction of dominant-lethal Mut. Res, 341 (1994) 109-131 mutations with triethylenemelamine in male mice. Genetics, 77: 753-763.

McConnel EE Huff J (1991) Toxicology and carcinogenisis studies on two grades of pentachlorophenol in B6C3FI mice. Fund. Appl ,Toxicol. 17519-532

Mc Mohan, RM., E R. Witkus and C.A Berger (1975) Some cytological characteristics of 2, 4-D on Pisum sativum (abstract No.243). Proc. Indian Sci. Acong (Bot.) 62-122.

McConnachie PR, Zahalsky AC (1991) Immunological consequences of exposure to pentachlorophenol. Arch of environ health, 46:249-253.

McConnell EE Huff JEM., Peters AC. Persing (1991) Toxicology and carcinogenesis studies of two grades of pentachlorophenol in B6C3F1 mice. Fundamental and applied toxicology, 17:519-532.

Mitelman F (1994) Catalogue of chromosome aberrations in cancer 5th edition ,New York Wilay.

*Meistrich, M.L., L.S. Goldstein A.J. Wyrobek (1985) Long-term infertility and dominant lethal mutations in male mice treated with adriamycin. Mut. Res: 152: 53-65.

*Meistrich, M.L., M. Finch, M.F. Da Cunha, U. Hacker W.W. Au (1982) Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. Cancer Res, 42: 122-131.

*Meyne, J. M.S. Legator (1983) Clastogenic effects of transplacental exposure of mouse embryos to nitrogen mustard or cyclophosphamide. Terat. Carcin. Mut, 3:281-287.

*Miltenburger, H.G., G. Engelhardt, G.Rohrborn (1981) Differential Chromosomal damage in Chinese hamster bone-marrow cells and in spermatogonia after mutagenic treatment. Mut. Res, 81: 117-122.

*Miltenburger, H.G., H. Trager, I.-D. Adier, A. Schmaltz, R. Rathenberg, H. Schwegler, I.H. Pawlowitzki, C. Nautsch and D. Muller (1978) The effect of isoniazid (INH) on Chinese hamster and mouse spermatogonia. Hum. Genet, 42: 44-49.

Ministry of Agriculture, Forestry and Fisheries, Agricultural Production Bureau, Japan, Good Laboratory Practice Standards, 59 NohSan No. 3850, January 1980, in : Agricultural Chemical Laws and Regulations , Japan (II), Society of Agricultural Chemical Industry, Tokyo, Japan, 1985, pp. 56-71.

*Mitchell, I.G., P.A. Dixon D.J. White (1981) Analysis of in vivo results, of cyclophosphamide-induced chromosomal damage in mammals from sensitivity and Statistical aspects. J. Toxicol. Environ. Health, 7: 585-592.

*Moreland, P.M., C.W. Sheu, J.A. Springer S. Green (1981) Effects of prolonged chemical treatment with cyclophosphamide and 6mercaptopurine in the dominant lethal test system. Mut. Res, 90: 193-199.

Moriya, M., T. Onta, K. Watanabe, T. Miyazwa, K. Kato, Y. Shirasu (1983) Further mutagenicity on pesticides in bacterial reversion assay systems. Mut. Res. 116 : 185-216.

*Moutschen, J. (1969a) Mutagenic effect of isopropyi methane sulphonate in mouse. Experientia, 25: 1337-1338.

*Moutschen, J. (1969b) Mutagenesis with methyl methanesulfonate in mouse. Mut. Res, 8: 581-588.

Muhling G., N. J. Vant Hof, G. B. Wilson, B. H. Grigslay (1960) Cytological effects of herbicides substituted phenols. Weeds, 8: 173-181

Mulcahy , M.T.(1980) Chromosomal aberration and agent orange Med. J. Aust 15: 573-574.

Muller,H (1928) Proc.Natl.Acad. Sci.US.14: 714.

*Mustonen, R., E.Glovaar, A.Zilling, K. linnainmaa, H. Vainio (1989) Effects of commercial chlorophenate, 2,3, 78-T4 CDD and pure phenoxyacetic acid on hepatic peroxisomes proliferation, xenobiotic metabolism and sister chromatid exchange in rat. Arch. Toxicol. 63:203-208.

Mustonen, R., J.Kangras, P. Vuojolaht, K. Lianainmaa (1986) Effects of phenoxyacetic acids on theinduction of chromosome aberrations in vitro and in vivo. Mutagenesis1: 24-25.

National Toxicology Programme (NTP) (1990) Toxicology and Carcinogenesis studies of 2,6 Xylidine (2,6 Dimethylanilene) (CAS No. 87-62-7) in Charles River CD Rats (FeedStudies) Technical Report Series No. 278 NIH Publication No. 90-2534. US Department of Health and Human Services, Public Health Service, National Institute of Health Research. Triangle Park NC.

National Toxicology Programme (NTP) (1989) Toxicology and Carcinogenesis Studies of Two Pentachlorophenol Technical Grade Mixtures (CAS No. 87-86-5) in B6C3F1 Mice (Feed Studies) Technical Reports Series NO. 349. NIH Publication NO. 89-2804. US Department of Health Service , National Institute of Health Research Triangle Park, NC.

*Nagao, T. (1987) Frequency of congenital defects and dominant lethals in the offspring of male mice treated with methylnitrosourea. Mut. Res, 177: 171-178.

Nagy, Z. S., I. Mile and F. Antoni (1975) The mutagenic effect of pesticides on *Escherichia coli* W P_2 try, Acta Microbiol. Acad. Sci. Hung 22, 309-314.

Naito S Y. Ono.Y. Ono I. Somiya.S. Mohri Ozone (1994) Role of active oxygen species in DNA damage by pentachlorophenol metabolites. Mut. Res, 310:79-88.

National Cancer Institute (1979) Bioassay of 2,46-trichlorophenol for possible carcinogenicity. Washington, DC, US Department of Health, Education and Welfare (Technical Report Series No. 155; DHEW Publication No. (NIH) 79-1711).

National Research Council Canada (1978) Phenoxy Herbicides –Their Effects on Environmental Quality with Accompanying Scientific Criteria for 2, 3,7, 8

Tetrachlorodibenzo-p-Dioxins (TCDD). Subcommittee on pesticides and relative compounds, WRC.Associate, Committee on Scietific Criteria for Environmental Quality, Ottawa, Canada.

National Intitute for Occupational Safety and Health (1989) Cincinnati ,A G 6825000.

Nishimura, N., H. Nishimura H. Oshma (1982) Survery of mutagenicity of pesticides by the Salomenella microsome test. AIDZA, 10: 305-312.

*Oakberg, H.F.- C.D. Crosthwait G-D. Raymer (1982) Spermatogenic stage sensitivity to 6mercaptopurine in the mouse, Mut Res, 94: 165-178.

Obe,G.,A.T.Nalarajf, Palatti (1982) Role of DNA double strand breaks in the formation of radiation induced chromosome aberrations. Prog.Mut.Res, 40:1-9.

OECD, (1983) Genetic toxocology,micronucleus test in; OECD Guidelines for testing of chemicals. pp 1-6.

Ohono, S. C., Stenius, E. Faisst, M. T. Zenzees (1965) Post Zygotic chromosomal rearrangements in rainbow trout (Salmo Irideus Gibbons). Cytogenetics . 4: 161-162.

Oehlkers, F (1943) Indukt.Abstam,u.Verenbungslenre 81,313.

Orser CS, Lange CC (1994) Molecular analysis of pentachlorophenol degradation. Biodegradation, 5:277-288.

Orser, C., S Lange C. C (1994): Molecular analysis of Pentachlorophenol degradation , Biodegradation, 5: 277-288.

*Oud, J.L. P.W.J. Peters (1978) A sequential screening of the cytogenetic damage induced by triaziquone, Mut Res. 54:175-184.

*Partington, M. H. Jackson (1963) The induction of dominant lethal mutations in rats by alkane sulphonic esters, Genet. Res, 4: 333 345.

Pavlica,M., g.I.V.Klobucar, N.mojas, R.Erben D.Papes (2000) Detection of DNA damage in heamatocytes in zebra mussel using comet assay Mut.Res,419:209-214.

Pavlica M., G.T.I.V. Klobucar W.Velma,R.Erben,D.Papes (2002) Detection of micronuclei in heamatocytes of zebra mussel and great ramshorn snail exposed to pentachlorophenol. Mut.Res.,465:145-150.

Pavlica, M.,J.Vasilevska, D.Papes (1999) Genotoxicity of Pentachlorophenol Revealed by Allium chromosome aberration assay. Acta.Biol , 40:85-95.

Pavilica M., D.Papes, B.Nagy (1991) 2,4-D acid causes chromatid and chromosomal abnormalities in plant cells and mutations in cultures mammalian cells. Mut. Res , 263:77-81.

*Pecevski, J., N. Maric, N. Savkovic, D. Radivojevic S. Green (1978) An Analysis of meiotic chromosomes of inbred male mice and their *Fl* sons after long-term treatment of sires with triethylenemelamine. Mut Res. 54: 55-60.

*Petersen, K.W.. M.S. Legator F.H.J. Figge (1972) Dominant-lethal effects of cyclohexyl amine in C57BL/FE mice. Mut . Res, 14: 126-129.

Pilinskaya, M.A (1974) The cytogenetic effects of herbicide 2,4-D on human and animal chromosomes. Tsitol .Genet 8: 202-206.

Pohjanvirta R, Tuomisto J (1994) Short-term toxicity of 2,3,7,8-tetrachlodibenzo-p-dioxin (TCDD) in laboratory animals: effects, mechanisms and animal model. Pharmacological reviews 46:485-549.

Pohlheim, E., F. Pohlheim,G.Guenther (1977) Mutagenicity testing of herbicides with a haploid pelangonium. Mut. Res, 46 232

Polakova, H. M. Vargova (1983). Evaluation of mutagenic effects of decametrin . Cytogenetic analysis of bone marrow. Mut. Res, 120:167-171.

*Poma, K., N. Dcgraeve, M. Kirsch-Volders C. Susanne (1981) Cytogenetic analysis of bone marrow cells and spermatogonia of male mice after in vivo treatment with arsenic. Experientia, 37: 129-130.

*Poorman-Allen. P., L.C. Backer, I.-D. Adier, B. Westbrook-Collins, M.J. Moses, J.W. Alien (1990) Bleomycin effects on mouse meiotic chromosomes. Mutagenesis, 5:573-581.

Rabbits (1994) Chromosomal translocation in human cancer. Nature, 372:143-149.

*Ramaiya, L.K., M.U. Pomerantseva G.A. Vilkina (1980) Comparative effectiveness of the use of various tests for the determination of Research 34} (1994) 109-131: 127 mutagenicity of certain factors in mammals. 1.

*Ramaya, L.K. M.D. Pomerantseva (1977) Investigation of cadmium chloride mutagenic effect on germ cells of male mice, Sov. Genet, 13: 43-46.

Rao, B. V., B. G. S. Rao and C. B. S. E. Sharma (1990) Unpublished data in Genetic toxicology pesticides in high plant systems, Sharma, C. B.S. R. and N. Pannear Sharma. Crit. Rev. Plant Sci, 9, 409-442.

Rao, K.S., J.D. Burek, F.J. Murray, J.A. John, B.A. Schwetz, TJ. Bell. W.J. Potts and C.M.Parker (1990) Toxicologic and reproductive effects of inhaled I,2-dibromo-3-chloropropane in rats.Fund. Appl. Toxicol, 3: 104-110.

Rasmuson B., Svahlin, H (1978) Mutagenicity tests of 2,4-dichlorophenoxyacetic acid and 2,4-5,trichlorophenoxyacetic acid in genetically stable strains and unstable strains of *drosophila melanogaster* in chlorinated Phenoxyacetic acids and t 4cdd Dioxins C Ramel (Eds) Swedish Naturalresearch Council: Stockholm, pp. 190-192.

Rasmusson, B., H. Persson Svahlin (1977) Mutagenicity tests of 2,4-D and 2,4,5 T in genetically stable and unstable strains of *Drosophila melanogaster* , conference on "Chlorinated phenoxyacids and their Dioxins. Mode of action health risks and environmental effects" The Royal Swedish Academy of Sciences, Stockholm, 6-10.

*Rathenberg, R. (1975) Cytogenetic effects of cyclophosphamide on mouse *spermatogonia. Hum Genet* , 29: 135-140.

*Ray, V.A., H.E- Holden, D.S. Salsburg, J.H. Ehis Jr., L.J. Just M.L. Hyneck (1974) Comparative studies of ethyl methanesulfonate-induced mutations with host-mediated, dominant lethal, and cytogenetic assays, Toxicol.Appl. Pharmacol. 30, 107-116.

Ramel, C., J. Magnusson (1979) Chemical induction of non disjunciton in Drosophila. Environ Health Perspect. 31: 59-66.

Regal, J. D., R. D. Synder. A. A. Francis (1983) Ultraviolet and chemically induced damage in the DNA of fibroblasts derived from two closely related species of marine fishes. Aquat. Toxicol. 4: 181-188.

Roberts, G.T. M.J. Rand (1978) The dominant lethal effects of some ergot alkaloids. Mut Res. 50: 317-325.

Reigner BG, Bois FY, Tozer TN (1993) Pentachlorophenol carcinogenicity: extrapolation of risk from mice to humans. Human experimental toxicology, 12:215-225.

Reigner BG, Rigod JF, Tozer TN (1992b) Disposition, bioavailability, and serum protein biding of pentachlorophenol in the B6C3F1 mouse. Pharmac. Res, 9:1053-1057.

Reingner BG, Bois FY, Tozer TN (1992a) Assessment of pentachlorophenol exposure in humans using the clearance concept. Human experimental toxicology, 11:17-26.

Roberts, F. L (1964) J. Morph., 115-401.

*Roberts, G.T., F.M. Johnson, H.V. Mailing R.K. Sharma (1979) Action of n-isopropyl-alpha-(2-methylhydrazmo)-p-toluamide hydrochloride (procarbazine hydrochloride) in the germ tissue of mice: dominant lethal effects, Arch. Toxicol, 41:287-294.

*Rohrborn, G (1965) Die mutagene wirkung vontrenimon bei der mannlichen maus, Humangenetik, 1: 576-578.

*Rohrborn, G. I. Hansmann (1971) Induced chromosome aberrations in unfertilized oocytes of mice. Hum .Gent. 13:184-198.

*Rohrborn, G., 0. Kuhn, 1. Hansmann K.Thon (1971) Induced chromosome aberrations in early embryogenesis of mice. Humangenetik, 11: 31 6-322.

*Rohrborn, G., V- Sezer, I.-D. Adier, A. Schmaltz, I.H. Pawlowitzki and C. Nautsch (1978) Effects of isoniazid (1NH) on the oogenesis of mice.Hum. Genet-42: 55-58.

*Rohrborn, G-A. Basler (1977) Cytogenetic investigations of mammals. Comparison of the genetic activity of cytostatics in mammals. Arch. Toxicol, 38: 35-43.

Rowley, J.D (1983) Human oncogene locations and chromosome aberrations. Nature, 27: 290-291.

*Russell, L.B. P.R. Hunsicker (1987) Study of the base analog 6-mercaptopurine in the mouse specific-locus test. Mut Res, 176: 47-52.

*Russell, L.B., P,R. Hunsicker N.L.A. Cacheiro (1986) Mouse specific-locus test for the induction of heritable gene mutations by di bromochloropropane(DBCP). Mut. Res, 17:161-166.

*Russell, L.B., P.B. Selby, E. Von Halle, W. Sheridan L. Valcovic (1981) The mouse specific-locus test with agents other than radiation. Interpretations of data and recommendations for future work. Mut Res, 86:329-354.

*Russell, L.B., R.B. Dimming P.R. Hunsicker (1984) Specific-locus mutation rates in the mouse following inhalation of ethylene oxide, and application of the results to estimation of human genetic risk. Mut Res, 129: 381-388.

*Russell, L-B., P.R. Hunsicker, N.L.A, Cacheiro W.M. Generoso (1991) Induction of specific-locus mutations in male germ cells of the mouse by acrylamide monomer. Mut Res, 262. 101-107.

*Russell, W.L (1972) Radiation and chemical mutagenesis and repair in mice, in: R.P. Beers, R.M. Herriot R.C. Tilghman (Eds.),Molecular and Cell Repair Process, John Hopkins Univ. Press, Baltimore, pp. 239-247.

*Russell, W.L. (1982) Factors affecting mutagenicity of ethylnitrosourea in the mouse specificlocus test and their bearing on risk estimation, in; T. Sugimura, S. Kondo and H. Takebe (Eds.), Environmental Mutagens and Carcinogens, Alan R. Liss. New York, pp. 59-70.

*Russell, W.L., L.B. Russell. (1978) Use of the mouse specific-focus method to quantify the gene mutation hazard from mutagens associated with non-nuclear energy technologies.ERA three-vear summary, EMIC No. 31729. 18 pp.

*Russell, W.L., E.M. Kelly, P.R. Hunsicker, J.W.Bangham, S.C. MadLiux, E.L. Phipps (1979) Specific-locus test shows ethylnitrosourea to be the most poler.: mutagen in the mouse, Proc. Natl. Acad. S.-L. USA, 76: 5818-5819.

*Russell, W.L., P.R. Hunsicker (1983) Extreme sensitivity of one particular germ-cell stage in male mice to induction of specific-locus mutations by methylnitrosourea, Environ, Mol. Mut, 5: 498.

*Russell, W.L.. P.R. Hunsicker, G.D. Raymer, M.H. Steele. K.F. Selzner , H.M. Thompson (1982) Dose-response curve for ethylnitrosourea-induced specific-locus mutations in mouse spermatogor.ia, Proc. Natl. Acad. Sci, 79: 3589-3591.

S Searle, A.G., D.A. Stephenson (1982) An in vivo method for the detection of somatic mutations at the cellular level in mice. Mut Res, 92: 205-215.

Sai-Kato K Umemura T, Takagi, A, Hasegawa R, Tanimura A Kurokawa (1995) Pentachlorophenol-induced oxidative DNA damage in mouse liver and protective effect of antioxidants. Food chem. and toxicol, 33:877-882.

*Saito-Suzuki, R., S. Teramoto, Y. Shirasu (1982) Dominant lethal studies in rats with1,2-dibromo-3-chloropropane and its structurally related compounds,. Mut.Res, 101:321-327.

Salkinoja, P-Salonen., M.S. et al (1989) Clean up of old industrial sites . Advances in applied biotechnology. 4:347-367.

Saracci R Kogevines M. Bertazzi PA , Bueno de. Mesquita BH, Coggon D, Green LM, Kauppinen T (1991) Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. Lancet, 338:1027-1032.

Schmid, W (1976) In : A. Hollander (Ed), Chemical Mutagens, Principles and Methods for their Detection, Vol 4, Plenum Press, New York, pp. 31-33.

Schop, R.N., M.H Hardy , M.T.Goldberg (1990) Comparision of accurity of topically applied pesticides and herbicides.2,4- D in two short term in vivo assays of genotoxicity in the mouse. Fund . Appl . Toxicol.15:666-675.

*Schreiner, C.A., J.R. Steelman (1977) Oral triethylenemelamine: effects in the dominant lethal assay for mutagenicity, Toxicol- Appl. Pharmacol, 42: 487-495.

Schwetz B.A., J.F, Quest, P.A. Keeler (1978) Results of two-year toxicity and reproduction studies on pentachlorophenol in rats. In: Ranga Rao K, ed. Pentachlorophenol. New York, NY, Plenum Press, pp.301-309.

Schwetz BA, Keepler PA, Gehring PJ (1974) The effect of purified and commercial grade pentachlorophenol on rat embryonal and fetal development. Toxicol and Appl Pharmacol, 28:151-161.

Schmid, W (1975) The micronucleus test .Mut. Res,31:9-15.

Seiler, J.P (1979) The genetic toxicology of phenoxyacids other then 2,4,5-T.

Seiler J.P (1991) Pentachlorophenol. Mut Res: 257:27-47.

*Seinenov, K.K., A.M. Malashenko (1979) Assessment of the contribution of thiophos phamide-induced chromosomal aberrations to preimplantation embryonic mortality in mice, yull. Exp. Biol. Mod. (USSR) 88, 1074-1076

*Semenov, K.K. A.M. Malashenko (1981) Appearance of dominant lethal mutations during early embryogenesi.s of the mouse, Sov. Genet, 17:309-313.

*Semenov, K.K., A.M. Malasbenko (1977) The search for translocation-heterozygous female mice among the progeny of males treated with M.D. Waters el al. / Mutation semisterility in male progeny of male mice treated with the chemical mutagen TEPA. Toxicol. Appl. Pharmacol, 19: 134-146.

*Setnikar, I., M.J. Magistrclii, M. Veronese (1976) Mutagenicity studies on nifurpipone and nitrofurantin. Proc. Eur. Soc. Toxicol, 17: 405-412.

*Sharma, R.K., G.T. Roberts. F.M. Johnson, H.V. Mailing (1979) Translocation and sperm abnormality assays in mouse spermatogonia treated with procarbazine. Mut. Res, 67: 385-388.

*Sherman, H., R. Culik, R.A. Jackson (1975) Reproduction, teratogenic, and mutagenic studies with benomyl. Toxicol.Appl. Pharmacol, 32:305-315.

Shirasu, Y (1975) Significance of mutagenicity testing on pesticides.Environ. Qual . Saf 4 : 226-2231.

*Shelby, M.D., K.T. Cain, C.V. Cornett, W.M.Generoso (1987) Acrylamide: Induction of heritable translocations in male mice. Environ.Mol. Mut, 9: 363-368. . Shelby, M.D., K.T. Cain, LA. Hughes, P.W. Braden, W.M. Gencroso (1986) Dominant lethal effects of acrylamide in male mice. Mut Res, 173: 35-40.

*Sheu, C.W., F.H. Moreland (1983) Detection of dominant lethal mutation in mice after repeated low-dose administration of 6-mercap topurine, Drug Chem. Toxicol, 6: 83-92.

*Sheu, C.W., F.M. Moreland. E.J. Oswald, S.Green, W.G. Flamm (1978) Heritable translocation test on random-bred mice after prolonged triethylenemelamme treatment.

*Shiraishi, Y. (1978) Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice. Mut Res, 57:313-324.

*Shively, C.A., D.M. White, J.L. Blauch, S.M.Tarka Jr. (1984) Dominant lethal testing of theobromine in rats. Toxicol. Lett, 20: 325-329.

Shyama, S. C., M. Abdul Rahiman, E. C. Vijayalaxmi (1991). Genotoxic effects of Anovlar 21, oral contraceptive, on mouse bone marrow. Mut. Res., 260: 47-53.

Sidrov V.P., K.D Mukhamedshin V.A Popara (1988) Mutagenic effects of 2,4-D sodium salts in two successive cell divisions. Tsit genetika 22(2) 37-40.

Simmon, V.F., K,Kauhanen, R.D.Tardiff (1977) Mutagenic activity of chemicals identified in drinking water in :Progress in oecclic Toxicology , vol 2, D. Scott, B.A Bridges, F.H Sodels (Eds) Elsievier /North holland: Amsterdam, pp.249-258.

Simmon, V.F, K. Kauhanen (1978) in vitro microbilogical mutagenicity assays of pentachlorophenol SRI Project LSU-5612, SRI International Menlo Park, CA.

Singh B.D, R.M.Singh, R.B. Singh, Y. Singh (1977) Reduction in anaphase bridge frequency by 2,4-D in irridiated abnormal wheat (*triticum aestivum*) root tips. Indian Journal.Expt .biol,526.

Singh B.D., B.I. Harvey (1975) Does 2,4-D induce, mitotic irregularities in plant tissue cultures?. Experientia, 31: 785-787

Soarsa, M., Wilbourn, J.Vainio, H. Magee, P.N. Mc Gregor, D.B.Mc Michael, A.J (Eds) (1992) Mechanics of carcinogenesis in RISK identification. Lyons: Internat Agen for Res on Canc, 543-554.

Sognier, M.A. and W. N. Hittelman (1986). Mitomycine induced chromatid breaks is HeLa cells; a consequence of incomplete DNA replication. Cancer Res, 46: 4032-4140.

Solomon M.F., J.A.Heedle, M.Katz.(1991) Mutagenicity activity of 41 compounds in the in vivo micronucleus assay. In: F.J.de Serres and J.ashby (Eds) Progress in mutation Research Vol.1 , evaluating short term tests for carcinogens. Reports of the International Collabration Programme Elseiver, New York pp.686-697.

Speit, G., K. Mehnat and W. Vogel (1987). Induction of endoreduplicaton by hydrazine in Chinese chamber V19 Cells and reduced incidence of sister chromatid exchanges in endoreduplicated mitosis, chromosomal, 89,79-84.

*Sram, R.J., M. Cerna , M. Kucerova (1976) The genetic risk of epichlorhydrin as related to the occupational exposure. Biol. Zentralbl, 95: 451-462.

*Sram, R.J., M. Cerna , M. Kucerova (1976) The genetic risk of epichlorhydrin as related tothe occupational exposure. Biol. Zentralbl, 95:451-462.

*Sram, R.J., V. Benes , Z. Zudova (1970a) Induction of dominant lethals in mice by TEPA and HEMPA.Folia Biologia, 16:407-416.

*Sram, R.J., Z. Zudova, V. Benes (1970b) Induction of translocations in mice by TEPA. Folia Bioiogia, 16: 367-368.

Stegeman J.J. J.J. Lech (1991) Cytochrome P450 monooxygenases system in aquatic species : carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ. Health Persp., 90: 101-109.

*Strekalova, Z.E., E.M. Chirkova, E.Y.A. Goiubovich (1975) Mutagenic action of ethylene oxide on the reproductive and somatic cells of male white rats. Toksikol. Nov. From. Khim. Veshchestvm 14: 11-16.

Styles , J.A (1977) Mammalian cell transformation in vitro. Br. J cancer ,37: 931-936.

Sudman, P.O., W.M. Generoso (1991) Female-specific mutagenic response of mice to hycanthone, Mut.Res, 246;31-43.

Suter, K.E. (1975) Studies on the dominant-lethal and fertility effects of the heavy metal compounds methylmercuric hydroxide, mercuric chloride and cadmium chloride in male and female mice. Mut Res, 30: 365-374.

Suter, K.E., W.M. Generoso (1976) Chemical induction of presumed dominant-lethal mutations in postcopulation germ cells and zygotes of mice. II. Sensitivity of different postcopulation-precleavage stages to three alkylating chemicals. Mut Res, 34: 259-270.

Sutou, S., K. Yamamoto, H. Scndota, M.Sugiyama (1980) Toxicity, fertility, tcratogenicity, and dominant lethal tests in rats administered cadmium subchronically. Ecotoxicol. Environ. Saf, 4:51-56.

Sutou, S. H. Y. Arai (1975) Possible mechanisms of endoreduplicaiton induction, Exp. Cell Res., 92, 15-22.

Suzuki Y., Y. Waga, J. Li, H. Sababa, K.Mo_2 2002, A. Takanasni, H. Shimizo (1989).Suzuki, Y., H. Shimizu. Y. Nagre, M. Ccumoto. H. Oconogi, M. Kodocura (1993). The micro nucleus test and erythorpoiesis : Effect of cobalt on the induction of micronuclei by mutagens,Environ. Mol. Mutagen. 22, 107-114.

Sykora, I., D. Gandalovicova (1978) Dominant-lethal assay of selected cytostatics, Neoplasma, 25:523-533.

Sykora. J. (1981) Dominant lethal test of 6mercaptopurine; dependence on dosage, duraration and root of administration. Neoplasma, 28:379-346.

Teaf, C.M., J.B. Bishop, R.D. Harbison (1990) Potentiation of ethyl methanesulfonate-mduced germ cell mutagenesis and depression of glutathione in male reproductive tissues by 1,2-dibromoethane. Terat. Carcin. Mut, 10: 427-438.

Teramoto, S., R. Saito, H. Aoyama, Y. Shirasu (1980) Dominant lethal mutation induced in male rats by l,2-dibromo-3chloropropane (DBCP), Mut Res, 77: 71-78. Testing. Toxicol. Appl. Pharmacol, 39: 549-552.

Tezuka, H., Y.F. Sasaki, M. Inoue, A. Uchida, M. Moriya, Y. Shirasu (1985) Heritable translocation study in male mice with trimethyl phosphate, Mut Res, 157: 205-213.
Thayer, P.S., CJ. Kensler (1973) Genetic tests in mice of caffeine alone and in combination with mutagen., Toxicol. Appl. Pharmacol, 25: 157-168.

Tomkins,D.J.,W.F.Grant(1976) Monitoring natural vegetation for herbicide induced chromosomal aberrations. Mut.Res. 36:73-84

Tripathy et al., (1993) Genotoxicity of 2,4-Dichlorophenoxye\acetic acid tested in somatic and germ line cells of Drosophila, Mut Res 273-243

Trukula, T.E., S.M.Jalal (1987) Induced clastogenecity in white rats by the herbicide 2,4-D. Cytologia 2: 275-281.

UBA (1996) Bewertung der Umweltgefahrlickeit ausgewahlter Altoffe durch das Umweltbundesamt, Teil II (Texte 38/96). Berlin, Umweltsbundesamt, pp. 59-69.

van Buul, P.P.W. (1984) Enhanced radiosensitivity for the induction of translocations in mouse stem cell spermatogonia following treatment with cyclophosphamide or adriamycin, Mut Res, 128: 207-211.

van Buul, P.P.W., J.H. Goudzwaard (1980) Bleomycin-induced structural chromosomal aberrations in spermatogonia and bone-marrow cells of mice, Mut Res, 69: 319-324.

Van Ommen, B.A.Adang.F.Muller,M.J.Vanbladeren (1986) The microsomal metabolism of Pentachlorophenol and its covalent binding to protein and DNA .Chem. Biol.Interact, 16:1-11.

Vartiainen T Lampi, J.P. Tumisto, J. Tuomisto (1995a) Polychlorodibenzo-p-dioxin and polychlorodibenzofuran concentrations in human fat samples in a village after pollution of drinking water by chlorophenols. Chemosphre, 30:1429-1438.

Vartiainen, T., et al (1995b) Polychloro dibenzo-p-dioxin and polychlorodibenzo-furan concentrations in lake sediments and fish after a ground water pollution with chlorophenols, Chemosphere, 30:1439-1451.

Vogel, E, J.L. R. Chandler (1974) Mutagenecity testing of Cyclamate and some presticides in *Drosophila melanogaster*, Experentia 30 : 621-623.

Wester,T.C et al (1993) Percutaneous absorption of pentachlorophenol from soil. Fundamental and applied Toxicology.20:68-71.

Waters, M.D., H.F.Stack, A.L.Brady, P.H.M.Lohman, L.Haroun H.Varnieo (1988) Use of computerized data testing and activity profiles of genetic and related effects in the review of 195 compounds.Mut.Res 205:294-312.

Wheng-Peng J., et al., (1982) Science 215: 181;Jprevention (Elsevier/ North-Hollanjd, Amsterdam,in press.

Wild,D., Bericht Zum Pestzid-Testprogram, in: Mutagenitatsprufung (1975) Kommission fur Mutagenitatsfragen der Deutschen Forsschungsge neinschaft, pp 16-35.

W., R. Rohrborn, P.Propping (1972) Mutagenicity studies with pesticides in host madiated assays and with the dominant lethal Test in mouse, Biol. Zentralbl, 91: 311-325

Waidyanatha S., P Lin, S.M Rappaport, (1994) Measurement of hemoglobin and albumin adducts of tetrachlorobenzoquinone. Chem. res in toxicol, 7:463-468.

Wang YJ., JK Lin (1995) Estimation of selected phenols in drinking water wit in situ acetylation and study on DNA damaging properties of polychlorinated phenols. Arch of environ contam and toxicol, 28:537-542.

Warers, M.D., H.F. Stack, A.L. Brady, P.H.M. Vainio (1988) Use of computerised data listings and activity. Profiles of genetic and related effects in the review of 195 compounds. Mut .Res, 205: 294-312.

Waters,M.D., Simmon, V.F, Milchell, A.d.Jorgenson t.a, Valencia, R (1980) An overview of short term tests for the mutagenic and carcinognic potential of pesticides. J.Environ. Sci. Health B15 (6), 969-709.

Wegler,R., L.Eue (1977) Chemieder Pflanzen Schutz and Schadlings,bekamp fungsmittel,Vol.5, Springer, Berlin , Heideiberg, New York, German.

Welsh J.J Collins, TFX, Black TN (1987) Teratogenic potential of purified pentachlorophenol and pentachloroanisole in subschronically exposed Sprague-Dawley rats. Food. chem and toxicol, 25:163-172.

WHO (1987) Pentchlorophenol. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 71).

Wobus, A., J. Schonech and R. Thieme (1978) The effect of the mode of administrations of nitrogen.

Woodruff, R.C., J.P.Phillips, D. Irwin (1983) pesticides induce complete and partial chromosome errors in screens with repair defective females of *drosophila melanogaste*r. Environ. Mut , 5:835-846.

Working, P.K., J.S. Bus, T.E. Hamm ST. (1985) Reproductive effects of inhaled methyl chloride in the male Fischer 344 rat. I. Mating performance and dominant lethal assay, Toxcol. Appl. Pharmacol, 77: 133-143.

Working, P.K., K.S. Bentley, M.E, Hurtt, L.M- Kathleen (1987) Comparison of the dominant lethal effects of acrylonitrile and acryi. amide in male Fischer 344 rats. Mutagenesis, 2: 215-220.

Yamamoto, K.I,, S. Hitotsumachi, Y. Kikuchi (1986) Occurrence .of premature chromosome condensation in mouse spermatogonia treated with busulfan.Cytogenet. Cell Genet, 43: 127-131.

Yang, J.H., P.T. Kostecki, E. J. Calabroceo, L.A. Baldwin (1990) Induction of peroxisome proliferation in rainbow trout exposed to cirrofibrate. Toxicol . Appl. Pharmacol. 104: 476-482.

Yuan,J.H.,T.J.Goehl,E.Murrill,R.Moore,Clark (1994) Toxicokinetics of Pentachlorophenol in the F344 rat .Gavage and dosed feed studies. Xenobiotica, 24:553-560.

Zahm, S.H et al (1990) Acase control study of non-Hodgkins lymphoma and the herbicide 2,4-D in eastern Nebraska Epidemiology 1:349-356.

Zeiger, E, B. Anderson, S. Hawort, T. Lawlor , . Mortelmans(1988). Salmonella mutagenicity tests: IV Results from testing of 300 chemicals Environ. Mol. Mutag 11 : 1-158.

Zetterberg, G (1977) Mutagenicity of chlorininated Phenoxyacetic acids in *Saccharomyces cerivisiae.* Abstract, and International conference on Environ. Mutagens, Edinburg, July 11-15

Zetterberg, G., (1978) Geneteic effects of phenoxy acids on microganismsin:Chlorinated Acids and Their Dioxins, C Ramel (eds) Swedish Natural Research Council: Stockholm, pp. 193-204.

Zetterberg, G., Busk, L, Elovson, R. Starec-Nordenhammas, I.H. Ryterman (1977) The influence of pH on the effectsof 2,4D (Na Salts) on the *Saccharomyces cerevisisiae* and *salmonella typhimurium.* Mut.Res, 42:3-18.

Zhurkov, V.S., R.I. Sram (1978) Dynamics of dominant lethal mutations in male mice exposed for different periods to cyclophosphamide in drinking water. Genetika, 14

Zhurkov, V.S., R.Y. Shram, A.M. Dugan (1983) Analysis of acrylonitrile mutagenic activity. Gig. Sanit 1, 71:72. 7

Zimmering S., J.M.Mason, R.Valencia R.C. Woodruff (1985) Chemical mutagenesis testing in drosophila II Results of 20 coded compounds, tested for the National Toxicology Programme. Environ. Mut, 7: 87-100.

Cross references*